In -Vitro Antioxidant and Nitric Oxide Scavenging Activity of *Oxalis dehradunensis* Raizada Plant Leaves

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Abstract- The current study was conducted to assess the in-vitro antioxidant activity of the methanolic extracts of leaves *Oxalis dehradunensis* Raizada, a plant with medicinal properties traditionally utilized in Indian folk drug. Reactive oxygen species (ROS) are known to induce oxidative stress, resulting in cellular injury and the development of multiple chronic disease. Therefore, the identification of natural antioxidants derived from plant sources are of considerable importance. Preliminary phytochemical screening of the extract indicated the presence of important biologically active compounds including alkaloids, flavonoid, tannin, phenolic compounds, terpenoids, and carbohydrate, while saponins were absent. The antioxidant potential of the extracts was evaluated using the DPPH assay radical scavenging and the nitric oxide scavenging activity was evaluated at different concentration levels (20–100 µg/mL), with ascorbic acid served as the standard comparison of reference. The results demonstrated that the extract showed notable free radical scavenging activity in a concentration de-pendent manner in both the assays. In the DPPH assay, the extract showed a maximum inhibition of 79.14% at 100 µg/mL, whereas ascorbic acid shows 93.18% inhibition. Similarly, in the nitric oxide scavenging assay, the extract exhibited 73.33% inhibition at the highest concentration, which was comparatively lower than the standard. Although the extract showed lower activity than ascorbic acid, it demonstrated considerable antioxidant potential, possibly attributed to the presence of phenolic and flavonoid compound. This result supports the traditional uses of the plant and suggest that it may serve as a promising organic source of antioxidants. Addi-tional studies are needed to isolate and characterize the active compounds responsible for this effect.

Key words: *Oxalis dehradunensis*, Antioxidant activity, DPPH assay, Nitric oxide scavenging.

Introduction

Reactive oxygen species (ROS) are generated within the body, and when their generation exceeds the capacity of intracellular defence systems to failure to neutralize them in a condition termed oxidative stress. This type of imbalance damage essential biomolecules like lipids, proteins, DNA, and RNA[1]. Such types of damage is linked to the development of various disorder such as cancer, oxygen toxicity, aging, lipofuscin accumulation, and liver damage[2-3]. Anti-

oxidants play a crucial role in safe-guarding against disease related to free radicals[4]. These compounds help in reducing oxidative stress by inhibiting oxidation and neutralizing reactive oxygen species. The antioxidant potential of plants are mainly attributed to the presence of phytochemicals, which interact synergistically to produce beneficial effects. In addition to their antioxidant properties, plant derived phytochemicals have shown the ability to interact with microorganisms in the environment, thereby inhibiting the growth of bacteria and fungi. Due to their antimicrobial activity and relatively low toxicity towards hosts cells, these com-pounds are considered promising potential sources for the development of novel antimicrobial agents[5]. Plants have served long as significant sources of medicinal agents for centuries. They are commonly employed in traditional systems of medicines and have contributed for the discovery of many effective drugs. Research in this field is largely based on traditional knowledge, especially the use of medicinal plants, which hold significant importance in folk medicine systems[6-7].

In India, *Oxalis dehradunensis* Raizada is widely acknowledged as a valuable medicinal plant due to its adaptability and broad range of physiological activities. Commonly referred to as creeping wood sorrel, this naturally occurring plant is rich in essential constituents that contribute to maintainning normal health and well-being in humans[8]. The plant is a stemless herb without a rootstock, forming bulb-like ovoid structures (about 5 × 2 cm). It produces several basal stolons with small scales that develop into bulbils. Petioles grow up to 20 cm long, and the leaflets are nearly equal, obdeltoid, and deeply incised. The inflorescence is umbellate with 5–12 flowers, supported by peduncles up to 25 cm long. Bracts are small and ovate, while pedicels are slender and reach up to 2 cm. Sepals are oblong-lanceolate with faint veins. Petals are narrow, pink to reddish-purple with a green base, and quickly wither after flowering[9]. In traditional Indian folk medicine, many easily available plants are used for treating fever, pain, inflammation, and wound healing. *Oxalis dehradunensis* Raizada has been selected for the present study due to its medicinal importance. It is a very common weed found in warm regions of India as well as other parts of the world[10].

Commonly known as garden pink sorrel or broadleaf sorrel, this flowering plant classified under the family of Oxalidaceae. The plant is a perennial herb and is native to regions such as Mexico, Central or South America, parts of the southeastern world[11]. Chemical studies have identified the presence of fatty acids, glycolipids, neutral lipids, vitexin, and isovitexin in *Oxalis dehradunensis* Raizada. Phytochemical investigations also report compounds such as tannins, palmitic, linoleic, linolenic, and stearic acids. The methanolic extract of the plant contains proteins, amino acids, carbohydrates, glycosides, and volatile oils, along with tannins and calcium rich fiber. The leaves are known to possess tannins, citric acid, calcium oxalate, and various flavonoids including apigenin, quercetin, orientin, and vitexin. Due to the high oxalate content,

the plant exhibits a distinctly acidic taste[12].

Leaves part of *Oxalis Dehradunensis* Raizada

Material and Methods

For the in-vitro antioxidant and nitric oxide scavenging studies of *Oxalis dehradunensis* Raizada leaves, various chemicals and reagents were employed. In the DPPH radical scavenging assay, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), methanol, or ascorbic acid served as procured from the Shaila Enterprise, while all others reagents used were of analytical grade quality.

In the nitric oxide scavenging assay, sodium nitroprusside was utilized as a source for generating nitric oxide in solution, and the formation of nitrite was measured using Griess reagent at 546 nm. The plant extract and reagents were prepared using methanol or phosphate buffer (pH 7.4), depending on the requirement. All experimental procedures were carried out using standard laboratory apparatus such as glassware, pipettes, and cuvettes. Each experiment was carried out three times to ensure reliability and accuracy of the result. Distilled water was used for preparing all dilutions.

Collection- *Oxalis dehradunensis* Rai-zada plant leaves have been taken from the local area of Dehradun, Uttarakhand and were shade dried at

Authentication- Authentication of *Oxalic dehradunensis* Raizada was done by BSI, Dehradun, UK.

Plant extraction- Dried and powdered plant (50g) of *Oxalis dehradunesis* was extracted by using a Soxhlet apparatus, the sub-stance was neutralized with 100 ml of petroleum-based ether and extraction with 100 ml of methanol over the course of 24 hours was carried out. A rotating vacuum evaporator was used to dry the extract, which was be kept refrigerated until needed again.

In-vitro Antioxidant activities

Antioxidant Assay- The antioxidant activity of the plant extract was assessed using the DPPH radical scavenging method. Each experiment was performed three times and average values were taken for analysis. Additionally, the capacity of the extract to scavenge nitric oxide radicals was assessed. In phosphate buffer, sodium nitro prusside served as the source for nitric oxide generation and its activity was determined using Griess reagent.

DPPH Radical Scavenging Assay- The methanolic extract of the plant exhibited free radical scavenging activity. *Oxalis dehradunesis* leaves were evaluated. A 0.004% (w/v) solution was made using 95% ethanol. Stock solutions (10 mg/100 mL) of the plant extraction were prepared by dissolving the methanolic extract in 95% ethanol or distilled water as required. Different aliquots (2, 4, 6, 8, and 10 mL) were pipetted from the stock solution into individual test tubes. The volume in each test tube was then adjusted to 10 mL using the respective solvent to obtain concentrations of 20, 40, 60, 80, and 100 µg/mL. Each test tube was then treated with a freshly prepared solution of DPPH (0.004%

w/v). After an incubation period of ten minutes or absorbance was recorded at 517 nm using a spectrophotometer. Ascorbic acid was used as the reference standard, prepared in a similar manner using distilled water. A control sample containing the same reagents without extract was also maintained. The %age of free radical scavenging activity was calculated using the appropriate formula[13-15].

% radicals-scavenging= Absorbance of control - Absorbance of test Sample) X 100
(Absorbance of control)

Nitric Oxide Scavenging Assay-The leaf extract demonstrated nitric oxide scavenging activity assessed at concentrations of 20, 40, 60, 80 and 100 µg/mL, using ascorbic acid, the standard reference. Sodium nitroprusside (5mM) was incubated with various concentrations of the extract or standard in phosphate buffer (pH 7.4) at room temperature for 150 minutes. Following incubation, an equal vol. of Griess reagents was added to every reaction mixture to react with the nitrite generated from nitric oxide. The resulting pink-colored solution was analyzed spectrophotometrically at 546 nm. The % age inhibition of nitric oxide radicals was calculated by comparing the absorbance of the test's samples with that of a control solution lacking the extract.

% Inhibition = (Absorbance of control - Absorbance of test Sample) X 100
(Absorbance of control)

Formula for sample absorbance for % inhibition- Absorbance sample – Absorbance of Control X (1- % inhibition/100)

Results

Preliminary phytochemical investigation of *Oxalis dehradunensis* **Raizada -** The phytochemical testing of methanolic extraction of leave of *Oxalis dehradunensis* **Raizada** shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

Table-1 Result of phytochemical test

Tests	HAE
Tests for Alkaloid	
1. Mayer's Tests	+
2. Wagner's Tests	+
3. Hager's Tests	+
4. Dragendroff's tests	+
Tests for Saponins	
1. Foam test	-
Test for Flavonoid	
1. Alkaline reagent tests	+
2. Lead Acetate tests	+
Tests for Tannins	
1. Gelatin + extract	++
Tests for Phenolic compound	
1. Ferric chloride solution	+
Tests for Terpenoids	
1. Salkowski test	+
Tests for Carbohydrates	
Molisch test	++

+ represents presence; ++ represents present in more concentrations; - represents absence.

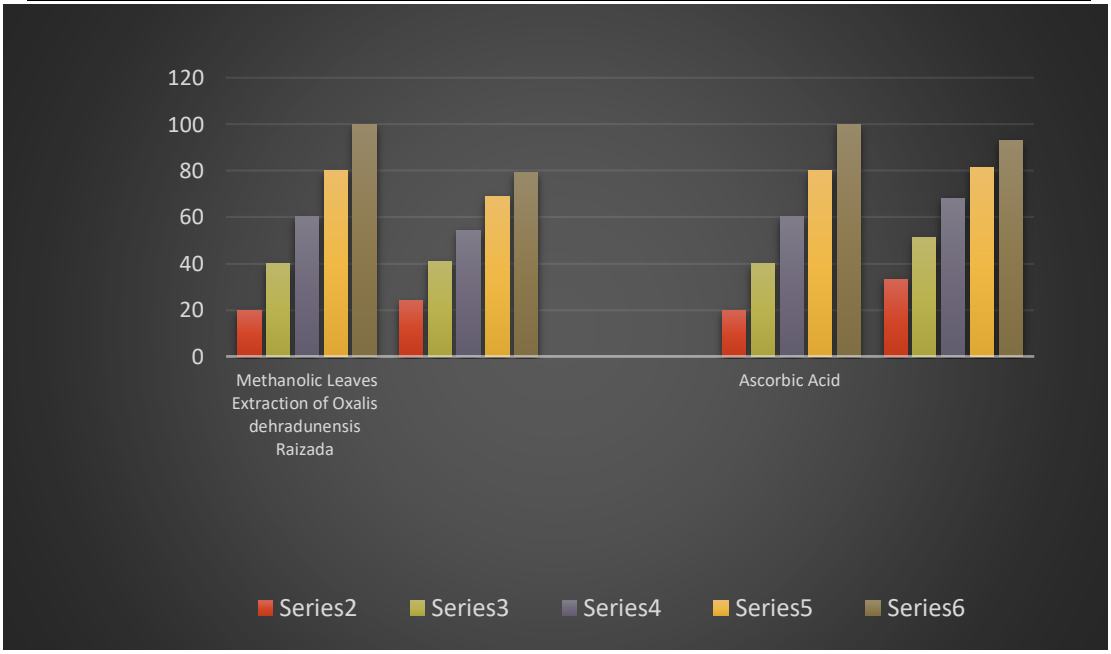
Phytochemical analysis- Initial phytochemical analysis of *Oxalis dehradunensis* Raizada leaves extracts revealed that the plant contains a wide range of bioactive compounds (secondary metabolite) Screening results indicated a strong to moderate presence of important constituents such as alkaloids, flavonoids, saponins, and carbohydrates (Table-I).

In-Vitro Antioxidant Activity

DPPH radical scavenging activity- DPPH radicals scavenging strength of *Oxalis dehradunensi* Raizada leaves extract at different two concentrations investigated in this current study was examined along with the standard antioxidants (Ascorbic Acid) in a similar concentration. *Oxalis dehradunensis* Raizada leaves extract (methanolic extracts) presented significant scavenging effects on DPPH free radicals in concentration dependent manner. When it was compared to the standard antioxidant used in the experiments, extracts showed fairly lesser DPPH free radicals scavenging potentials.

Table-2 Antioxidant activities of methanolic extracts of leaves parts of *Oxalis dehradunensis* Raizada by DPPH method

S.NO.	Methanolic Leaves Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	***DPPH % Inhibition***	***Concentrations (µg/mL)***	***Percentage Inhibition***
01	20	24.16	20	33.14
02	40	40.98	40	51.23
03	60	54.45	60	68.19
04	80	69.13	80	81.18
05	100	79.14	100	93.18

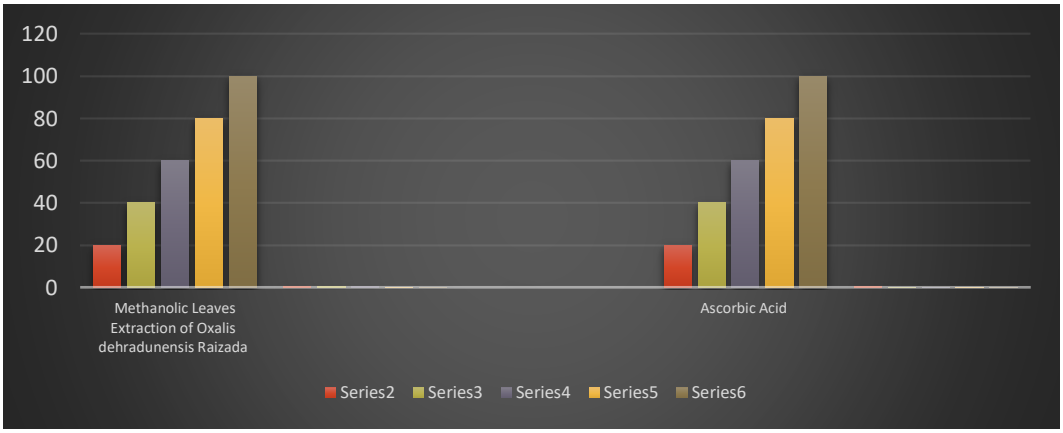


Graph -1 shows the % inhibition of DPPH radicals by extraction of *Oxalis dehradunensis* Raizada

Table-3 Antioxidant activities of methanolic extraction of leaves part of *Oxalis dehradunensis* Raizada by DPPH method

S.NO.	Methanolic Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	**Absorbance at 517nm**	***Concentration (µg/mL)***	**Absorbance at 517nm**
01	20	0.619	20	0.499
02	40	0.489	40	0.302
03	60	0.398	60	0.199
04	80	0.219	80	0.118
05	100	0.119	100	0.021

Graph -2 showing the absorbance of DPPH radicals by extracts of *Oxalis dehradunensis* Raizada



Nitric Oxide (NO) Scavenging Activities- In the current study, the nitric oxide radical scavenging activity of ***Oxalis dehradunensis*** **Raizada** leaf extract was evaluated at various concentrations or compared it with a standard antioxidant, ascorbic acid, at corresponding concentrations. The methanolic extract of *Oxalis dehradunensis* **Raizada** leaves demonstrated notable nitric oxide scavenging activity, which increased in a concentration-dependent way. However, when relative to the standard antioxidant applied in the study, the extracts exhibited comparatively low scavenging efficacy against nitric oxide radicals.

Table-4 Antioxidant activities of methanolic extraction of leaves part of *Oxalis dehradunensis* Raizada by Nitric Oxide (NO) scavenging method

S. no.	Methanolic Leaves Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	***Nitric Oxide % Inhibition***	***Concentrations (µg/mL)***	***Percentage Inhibition***
01	20	16.24	20	28.12
02	40	30.18	40	48.12
03	60	45.97	60	69.56
04	80	59.12	80	69.25
05	100	73.33	100	80.13

Graph- 3 shows the % inhibition of Nitric oxide radicals by extraction of *Oxalis dehradunensis* Raizada

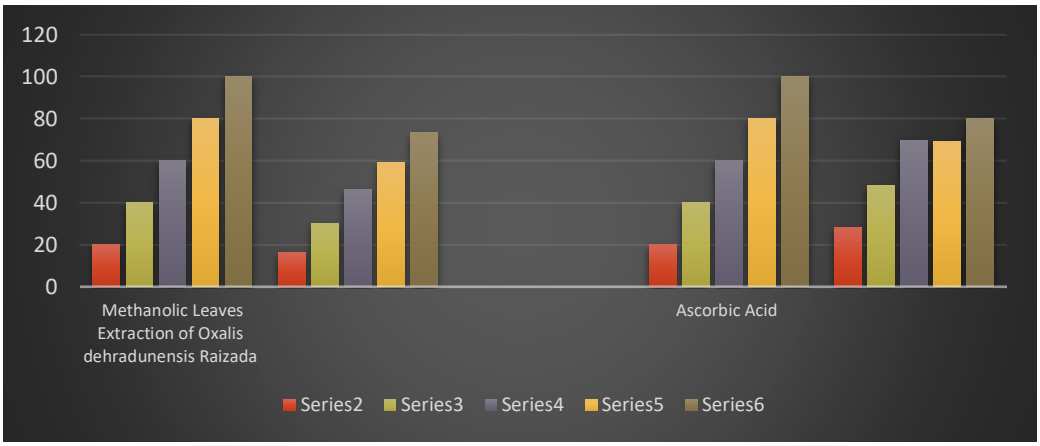
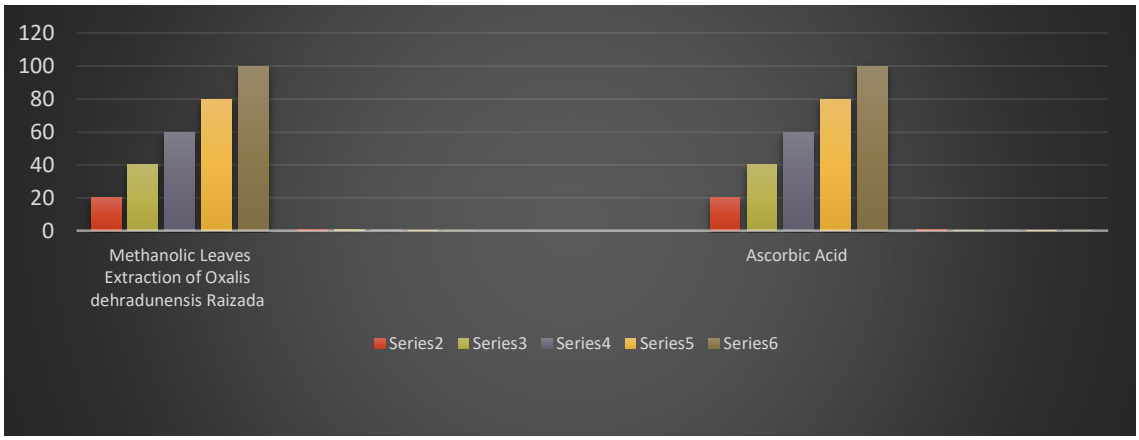


Table-5 Antioxidant activities of methanolic extraction of leaves part of *Oxalis dehradunensis* Raizada by Nitric Oxide (NO) Scavenging method.

S.NO.	Methanolic Leaves Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	**Nitric Oxide Absorbance at (546nm)**	***Concentrations (µg/mL)***	***Absorbance at (546nm)***
01	20	0.599	20	0.589
02	40	0.512	40	0.399
03	60	0.476	60	0.243
04	80	0.288	80	0.115
05	100	0.132	100	0.087

Graph -4 shows the % inhibition of Nitric oxide radicals by extraction of *Oxalis dehradunensis* Raizada



Discussion

In this current study, in-vitro anti-oxidant potential of the methanol used leaf extract of *Oxalis dehradunensis* Raizada was evaluated using both DPPH or nitric oxides scavenging assay. The finding revealed that the extracts exhibited considerable free radical sca-venging activities in both the methods, with effect increasing as the concentration rise. This suggested that

higher doses of the extract are more efficient in neutralizing reactive species. On comparison with the standard anti-oxidant, ascorbic acid, *Oxalis dehra-dunensis* Raizada extract showed com-paratively lower percentage inhibition across all tested concentrations. This could be attributed to the facts that plant leaves extracts contain a complex mixture of phytoconstituents, which may act synergistically but are generally less potent than isolated standard compounds under in-vitro conditions. Despite this, the extract demonstrated appreciable anti-oxidant activity, suggesting its potential as a natural antioxidant source.

In the DPPH assay, which evaluate the extract's hydrogen donation potential to stabilize free radicals, the extract achie-ved a maximum inhibition of about 79.14% at a conc. of 100 µg/mL, where-as ascorbic acid exhibited 93.18% inhibition at the same concentration. Similarly, within the nitric oxide scavenging test, the extract showed effective inhibition of nitric oxide radical, which are reported to play a role in oxidative stress or inflammatory processes.

However, the activity observed in the nitric oxide assay was slightly lower than that in the DPPH method, indicating that the extract may possess stronger hydrogen donating capacity than nitric oxide scavenging ability. These observations agree with earlier reports on plant derived antioxidants, where methanolic leaf extracts are known to be rich in phenolic and flavonoid compounds responsible for scavenging free radicals. The prog-ressive increase in activity with concentration further highlights the potential of *Oxalis dehradunensis* Raizada leaves as a promising natural anti-oxidant source.

Overall, although the extract showed lower potency compared to ascorbic acid, it still exhibited significant anti-oxidant and nitric oxide scavenging activities. These results support its tradi-tional medicinal uses and suggest the needs for further study to explore its healing potential.

Conclusion

The present investigation revealed that the leaves of *Oxalis dehradunensis* Raizada are rich in diverse second-ary metabolites. These phytoconstituents could serve as valuable sources of pharmacologically active compounds, suggesting that this plant species holds considerable potential for management of many long-term diseases. In addition, crude extract demonstrated promising antioxidant activity, thereby providing scientific support for its traditional medicinal use. However, further detailed studies are necessary to facilitate the development of novel antioxidant therapeutics.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Dastmalchi, K.; Dorman, H.J.D.; Kosar, M. and Hiltunen R. Chemical com-position and in vitro antioxidant eva-luation of a water-soluble Moldavian balm (Dracocephalum moldavica L.) extract. *Leben. Wiss. Technol.;* 2007; 40: 239–248.

2. Iyer, D. and Devi, P.U. Radio-protective activity of Murraya koenigii L. On cellular antioxidants in swiss albino mice. *J. Pharmaceut. Res.;* 2009; 2: 495-501.

3. Smerq, J. and Sharma, M. Possible mechanism of *Murraya Koenigi* and *Cinnamomum tamala* in Swiss albino mice with reference to antioxidant activity. *Int. J. Pharmaceut. Sci. Drug Res*.; 2011; 3: 260- 264.

4. Hou, W.C.; Lin, R.D. Cheng, K.T.; Hung, Y.T.; Cho, C.H.; Chen, C.I.; Hwang, S.Y. and Lee, M.H. Free radical scavenging activity of Taiwanese native plants. *Phytomedicine*; 2003; 10: 170-175.

5. Bruneton. *Phytochemistry of Medicinal Plants. J. Pharmacognosy; Lavoiser publishers France; 1995; $p_{p.}$ 265–380.*

6. Council of Scientific and Industrial Research (CSIR). *The Wealth of India: Raw Materials;* Vol. X; New Delhi; CSIR; 1976; $p_{p.}$ 175.

7. Croom, E.M. Documenting and evaluating herbal remedies. *Econ Bot.;* 1983; 37: 13–27.

8. Morris, R. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*; 1984; 11(1):47-60.

9. Jankovic, J.; Goodman, I. and Safirstein, B. et al. Safety and tolerability of multiple ascending doses of PRX002/RG7935, an Anti–α-Synuclein monoclonal antibody, in patients with Parkinson disease. *JAMA Neurol.;* 2018; 75:1206–14.

10. Savitt, D. and Jankovic, J. Targeting α-Synuclein in Parkinson's disease: Progress Towards the Development of Disease Modifying Therapeutics. *Drugs;* 2019; 79:797– 810.

11. Espay, A.J.; Hauser, R.A. and Armstrong, M.J. The narrowing path for nilotinib and other potential disease-modifying therapies for Parkinson disease. *JAMA Neurology*; 2019; doi:10.1001/jamaneurol.2019.3983. [Epubahead of print: 16 Dec 2019].

12. Ding, X. and Ren, F. Leucine-rich repeat kinase 2 inhibitors: a patent review (2014 present). *Expert Opin. Ther. Pat.*; 2020: 1–12.

13. Soares, J.R.; Dinis, T.C.P.; Cunha, A.P. and Almeida, L.M. Antioxidant activities of some extracts of Thymus zygi. *Free Radical Research*; 1997; 26:469–478.

14. Manian, R.; Anusuya, N.; Siddhuraju, P. and Manian, S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis*. *Food Chemistry*, 2008; 107:1000–1007.

15. Kaur, G.; Tirkey, N.; Bharrhan, S.; Chanana, V.; Rishi, P. and Chopra, K. Inhibition of oxidative Stress and cytokine activity by curcuminin amelioration of endotoxin-induced Experimental hepatotoxicity in rodents. *Clinical and Experimental Immunology*; 2006; 145: 313–321.