**American Journal of Nanosciences**
2022; 8(1): 1-7
http://www.sciencepublishinggroup.com/j/ajn
doi: 10.11648/j.ajn.20220801.11
ISSN: 2575-484X (Print); ISSN: 2575-4858 (Online)

# In-vitro Evaluation of Anti-oxidant and Anti-inflammatory Potential of Bio-synthesized Silver Nanoparticles from Endemic Medicinal Plant Species *Terminalia pallida*

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## To cite this article:

P. Rama Mohan, N. Savithramma. In-vitro Evaluation of Anti-oxidant and Anti-inflammatory Potential of Bio-synthesized Silver Nanoparticles from Endemic Medicinal Plant Species *Terminalia pallida*. American Journal of Nanosciences. Vol. 8, No. 1, 2022, pp. 1-7.
doi: 10.11648/j.ajn.20220801.11

**Received**: December 17, 2021; **Accepted**: February 8, 2022; **Published**: April 9, 2022

**Abstract:** *Background:* The Seshachalam hill ranges of Eastern Ghats are known for their endemic flora. There were seven endemic species situated here in which present plant species *Terminalia pallida (T. pallida*) is one, this Endemic semi-tree species has a great medicinal value; at the same time, due to the presence of different types of secondary metabolites, they easily form nanoparticles and exhibit certain high-quality characteristics after being combined with noble metals like silver. *Objective:* These Silver Nanoparticles (SNPs) display peculiar characteristics in their nano level which shows more effectiveness compared to their raw extracts, with negligible side effects. The present work shows *in vitro* antioxidant (DPPH radical scavenging assay) and anti-inflammatory (inhibition of albumin denaturation) applications of SNPs synthesized from leaf, bark, and fruit extracts. *Method:* Antioxidant activity was carried out by Burits and Bucar method with slight changes and about anti-inflammatory (inhibition of albumin denaturation) assay was carried out by Dunnet method. *Result:* In these experiments, the results obtained were positive. The result, when compared among the three parts, showed more positive results in antioxidant assay SNPs synthesized from fruit, and dominated results in the anti-inflammatory test SNPs synthesized from the bark. *Conclusion:* By studying the applications of SNPs it is confirmed that the biosynthesized silver nanoparticles (SNPs) are helpful in the production of various medicines hence SNPs can be recommended ingredients for biomedicine.

**Keywords:** *Terminalia pallida,* Endemic Medicinal Plant, Silver Nanoparticles, *in-vitro* Antioxidant, Anti-inflammatory Assays

## 1. Introduction

The human body for its health purpose needs antioxidants as supplements they are available naturally in beneficial phytochemicals present in plants [1]. Studies about plants proved that vitamins A, C, E, and phenolic compounds like flavonoids, tannins, and lignins all act as natural antioxidants [2]. Antioxidant defense systems can be noted as non-enzymatic and enzymatic, used by all aerobic organisms including humans to stop autoxidation or peroxidation [3]. Plants were the richest sources of most of the organic contents, minerals, other valuable combination of compounds and products all these help plants for leading a better life for years these compounds can cure many diseases caused by the pests, external damages, disturbances in the internal physiological process most probably conditions like stress caused by the high or overheat conditions. Generally, all plants have more or less certain medicinal properties and also consist of qualities like antioxidants, etc., The medicinal properties in a plant richer due to the presence of sundry display of secondary metabolites along with antioxidant potential [4]. It is also correct to say that every herbal drug might show a different combination of ingredients and shows accurate medicinal properties with the support of these antioxidants [5-8]. These antioxidants present in plants naturally to support plant life. These antioxidants

concentration is from low to high in different plant species depending upon the growing environmental conditions, their action depends upon the concentration and capacity, this can be measurable [9-11]. Plants having aromatic characteristics or medicinal properties are at present in highest usage as food and supplementary food because of their nutrient and medicinal values at the same time they are having antioxidant and medicinal importance [9-11]. To get rid of artificial or synthetic or chemical antioxidants these natural antioxidant products helping a lot [7-11]. The plants are having polyphenols and flavonoids as major constituents showing anti-oxidant and medicinal properties [10, 11]. To smother the redox reactions of free radicals in physiological systems these compounds are helping [8-11]. The value of a plant as a medicine, reaction property as well as a food can be identified with the help of the presence of antioxidant richness and in their absence identified the damage happened to biological molecules with different mechanisms [12-17]. It was also noticed that plant extracts showing antioxidant properties due to the presence of phytochemicals and they are having redox potentiality [18]. Plants generally produce neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. These can be cleared by the antioxidative activity of secondary metabolite phenols due to their redox nature it can be possible [19]. There were several researchers found that the concentration of total phenolic contents and antioxidative capacity have secured liaison [20-22]. The antioxidant activity shows synergistic and antagonistic effects due to the combination of different compounds. Phenols show a highly specialized character known as chelating the metals, this is due to the presence of hydroxyl and carboxyl groups, and they also show the ability to binding particularly iron and copper metals [23]. Antioxidants slow down the rate of oxidation of oil (caused by Reactive Oxygen Species ROS), or food lipids when present naturally or added artificially in foods [24, 25].

Infectious microorganisms such as bacteria, viruses, or fungi when invading the body; reside in particular tissues or circulating in the blood inflammation occurs [26-29]. Tissue injury, cell death, cancer, ischemia, and degradation when occurring to parts of a body the response shown by parts is known as inflammation [26, 30-35]. It was also defined that there were innate immune response and adaptive immune response were responsible in the formation of inflammation [26, 31, 35]. Inflammation is defined as a response showing by the living tissues when injured, in other words, Inflammation was also known that the lethal stimulus generally is given by the host against a damage-causing organism or a chemical and these stimuli are different for specific conditions [36]. The most important four indicators of inflammation were pain, redness, heat or warmness, and swelling. Injury to any part of the body the arterioles present around the tissue enlarges due to this circulation of blood raises at that injured part causing redness [37], and it leads to loss of function the increased blood circulation increases the formation of intracellular spaces due to these leukocytes, protein, and fluids move to inflamed regions of the injury [38, 39]. Inflammation is differentiated into two main types they were acute and chronic. Acute inflammation is the immediate harmful stimuli to injury of a body, whereas chronic inflammation is the inflammatory response after damage to the body. Inflammation is either acute or chronic inflammation. Acute inflammation may be an initial response of the body to harmful stimuli. In chronic inflammation, the inflammatory response is out of proportion resulting in damage to the body. In inflammation, pain and platelet aggregation are carried out by synthesis of prostaglandins, prostacyclins and thromboxanes for this an enzyme Cyclooxygenase (COX) are responsible [40]. The inflammatory response shows three important components [41] these components were having vasoactive substances [42] chemotactic factors [43, 44], degradativeenzymes and superoxides [3] and the neuropeptide, substance P [45]. Excessive activation of phagocytes, O-2, OH free radicals, and $H_2O_2$ non-free radicals species production takes place in a number of inflammatory disorders, these can be harmful to tissues by the action of powerful direct oxidation which takes by activating matrix metello proteinase damage in different arthritic tissues [46, 47].

# 2. Material and Methods

### *2.1. Collection of Plant Material*

Leaves, bark and fruits of *T. pallida* (*Terminalia pallida*) were collected from the Tirumala hills. The leaves and fruits were washed with tap water and then with distilled water to remove any foreign particles which can change the accuracy of all results in the primary, secondary as well as silver nanoparticles synthesis. The three parts were separately dried in a cool shade environment for 20 days after that those parts powdered stored in three airtight jars for future use.

There were various Chemicals, Reagents, Metals, and Glassware, etc. used for screening of phytochemicals, aqueous synthesis of SNPs and their Characterization, *in–vitro* antioxidant or DPPH scavenger activity, and *in–vitro* anti-inflammatory tests. For all activities and tests the plastic, glassware, and chemicals reagents were purchased from M/O. SR Scientifics, Indian Scientifics, and Bros Scientifics in Tirupati. Chemicals used for both the tests are 1, 1-diphenyl-1-picrylhydrazyl (DPPH), egg albumin, phosphate-buffered saline, and Diclofenac sodium.

### *2.2. Synthesis of SNPs from Leaf, Bark and Fruits of T. Pallida for In-vitro DPPH Scavenger Assay and In-vitro Anti-inflammatory Tests*

5gms of plant powders (leaf, bark and fruit) were taken into three separate conical flasks contatining 100ml of $DH_2O$ (double distilled water) and kept on a water bath at 80°C for 20 min and taken off from water bath and filtered with Whatman No. 1 filter paper. The collected aqueous filtrates of three samples were taken 5 ml each and mixed with 50 ml of $Ag(NO_3)_2$ solution and placed.

### *2.3. In-vitro DPPH Radical Scavenging Assay with SNPs Synthesized from Leaf, Bark, and Fruit from T. pallida*

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple-colored methanol solution of 1, 1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (25, 50, 75, and 100 µg/mL) in methanol was added to 4 ml of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. This assay was done according to a slightly modified method [48].

The ability of DPPH• radical scavenging assy (RSA) of SNPs can be calculated by using the standard formula:

$$RSA\% = (A0 - A1)/A0 \times 100$$

Here, A0 – the absorbance of the control, and A1 – the absorbance of the sample extracts.

### *2.4. Assessment of in Vitro Anti-inflammatory Activity by Inhibition of Albumin Denaturation with SNPs Synthesized from Leaf, Bark, and Fruit of T. pallida*

The protocol used for this was the "Dunnet" method with slight modifications and there are other protocols that are also present to do this testing [49]. The reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (pH 6.4), and 2 ml of varying parts of the leaf, bark, fruit of the test extract, and SNPs by which the concentrations (1mM) became Similar volume of double-distilled water served as control. The calculated standard drug diclofenac from the previous research articles was taken to its least concentration suitable to the 1mM SNPs which was 10 µg/mL [50-52]. Then the mixtures were incubated at 37°C ± 2°C in an incubator for 15 min and then heated at 70°C for 5 min. After cooling, absorbance was measured at 660 nm (UV Spectrophotometer 1800) used as blank. Diclofenac sodium was used as a reference and the percentage inhibition of protein denaturation was calculated by using the following formula [53].

Percentage inhibition = (Abs Control –Abs Sample) X 100 / Abs control of 3 replicates.

Statistical analysis: Results are expressed as Mean ± SD. The difference between experimental groups was compared by One Way Analysis of Variance (ANOVA) followed by Dunnet (DMDF) / Multiple comparison tests (control Vs test) using the software Graph Pad Instat.

***Table 1.*** *Antioxidant activity of different concentrated SNPs synthesized using leaf, bark and furit extract of T. pallida and their respective extracts.*

| T. pallida | 25 µg/mL | 50 µg/mL | 75 µg/mL | 100 µg/mL |
|---|---|---|---|---|
| Ascorbic acid | 48.26 ± 0.36 | 60.45 ± 0.20 | 77.34 ± 0.42 | 84.12 ± 0.16 |
| TPL Extract | 41.34 ± 0.15 | 45.50 ± 0.17 | 53.24 ± 0.22 | 62.18 ± 0.18 |
| TPL SNPs | 52.89 ± 0.24 | 59.37 ± 0.11 | 67.16 ± 0.12 | 74.24 ± 0.28 |
| TPB Extract | 39.16 ± 0.59 | 41.52 ± 0.24 | 54.46 ± 0.22 | 62.25 ± 0.11 |
| TPB SNPs | 41.25 ± 0.17 | 51.65 ± 0.15 | 64.29 ± 0.12 | 70.34 ± 0.13 |
| TPF Extract | 41.17 ± 0.24 | 48.24 ± 0.66 | 54.22 ± 0.65 | 60.06 ± 0.21 |
| TPF SNPs | 59.24 ± 0.21 | 70.11 ± 0.29 | 76.37 ± 0.30 | 80.20 ± 0.28 |

## 3. Result and Discussions

### *3.1. Antioxidant Activity (or) DPPH Radical Scavenging Assay*

DPPH radical scavenging assay of T. pallida leaf, bark and fruit extract and their extract mediated synthesized silver nanoparticles with different concentrations (25-100 µg/mL) results were respresent in Table 1 compare with standard Ascorbic acid. As a part of the procedure, the methanol extracts having polyphenols were screened for their antioxidant capacity by using this DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay. The same results were represented in the form of graphs; this provides information about the scavenging capacity difference among the parts of the tree. Leaf extract and SNPs along with standard ascorbic acid were taken in equal concentrations, the results show that the SNPs were shows increase steadily i.e., for least at 25 µg/mL it was 52.89 ± 0.24 for the highest concentration i.e., 100 µg/mL it shows the value 74.24 ± 0.28. When compared to the standard ascorbic acid in the least concentration of 25 µg/mL it was 46.42 ± 0.13 at the same concentration SNPs shows more activity with values 52.89 ± 0.24 than ascorbic acid.

Bark extract and SNPs along with standard ascorbic acid were taken in equal concentrations, the results show that the SNPs values were shows increase steadily i.e., for least at 25 µg/mL it was 41.25 ± 0.17, for the highest concentration i.e., 100 µg/mL it shows the value 70.34 ± 0.13.

Fruit extract and SNPs along with standard ascorbic acid were taken in equal concentrations, the results show that the SNPs values were shows increase steadily i.e., for least at 25 µg/mL it was 59.24 ± 0.21 for the highest concentration i.e., 100 µg/mL it shows the value 80.20 ± 0.28. When compared to the standard ascorbic acid in the least concentration of 75µg/mL, it was 77.34 ± 0.42 at the same concentration, SNPs shows the equal activity of ascorbic acid with values 76.37 ± 0.30.

The consideration of SNPs DPPH scavenging activity of

the leaf, bark, and fruit, fruit showing more free radical scavenging activity against DPPH. The values of fruit SNPs were high compared to the leaf and bark this confirms the active in DPPH scavenging activity of fruit SNPs were strong compared to the other two parts. In all the graphs the three extracts were used in the same concentration and that all shows antioxidant activity in a standard way with less effective. In the case of SNPs of three samples (TPL, TPB & TPF) were in the same concentrations performed activity differently at different concentrations.

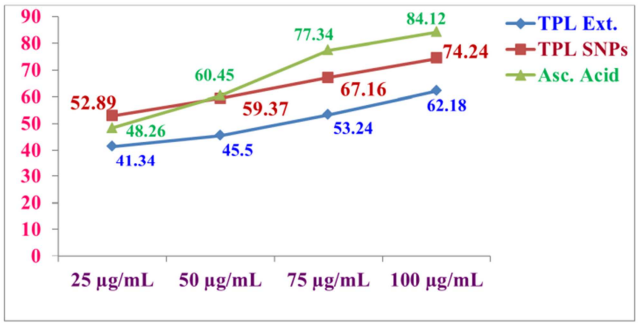


***Figure 1.*** *DPPH scavenging Assay and Evaluation of $IC_{50}$ of the Terminalia pallida Leaf extract and SNPs compared with standardascorbicacid. (Concentration in µg/mL on X-Axis DPPH % scavenging on Y-Axis).*

The DPPH / Ascorbic acid with the same concentration has shown the highest standard way in all the three samples. The SNPs from the leaf (Figure 1) at 25µg/mL shows good performance than ascorbic acid; at 50 µg/mL it performed nearly equal to ascorbic acid, but at 75µg/mL and 100 µg/mL its performance was decreased compared to the standard ascorbic acid.

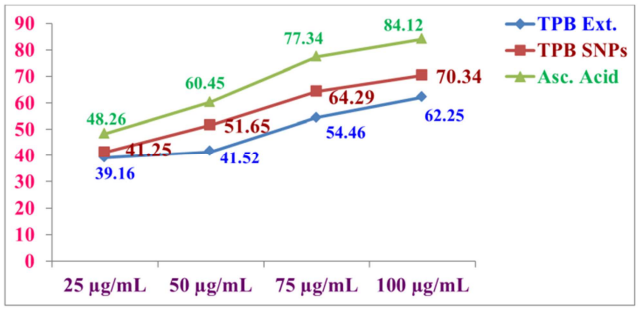


***Figure 2.*** *DPPH scavenging Assay and Evaluation of $IC_{50}$ of the Terminalia pallida bark extract and SNPs compared with standardascorbicacid. (Concentration in µg/mL on X-axis DPPH % scavenging on Y-axis).*

The DPPH / Ascorbic acid with the same concentration has shown the highest standard way in all the three samples. The SNPs from bark (Figure 2) from 25µg/mL - 100 µg/mL shows good performance but lesser than ascorbic acid.

The DPPH / Ascorbic acid with the same concentration has shown the highest standard way in all the three samples. The SNPs from fruit (Figure 3) at 75µg/mL shows good performance with nearly equal to ascorbic acid confirms the all the parts were showing good antioxidant activity.

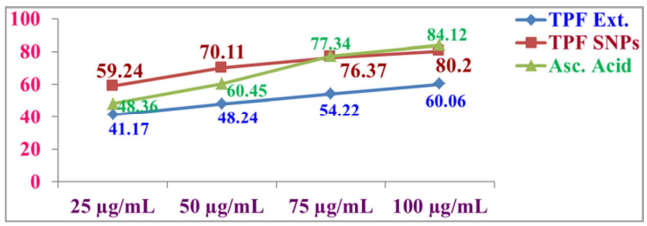


***Figure 3.*** *DPPH scavenging Assay and Evaluation of $IC_{50}$ of the Terminalia pallida fruit extract and SNPs compared with standardascorbicacid. (Concentration in µg/mL on X-axis DPPH % scavenging on Y-axis).*

The quality of the DPPH radical is stable when present in the methanolic solution. Antioxidants present in extract SNPs shows the scavenging activity with DPPH; this reduction of DPPH was monitored by the decreasing the absorbance to 517 nm not only that this symbolized that the purple color present in the initially in the solution turns to yellow at this point, but it was also confirmed that full amount of free radicals present were completely clocked by the antioxidants. The curves obtained by these calibrations from different concentrations in leaf, bark, and fruit extracts and SNPs the inhibitory concentration 50% value provides the information about the concentration of samples required to scavenge 50% of the DPPH free radicals [54]. While doing free radical scavenging activity of SNPs on DPPH radicals it was noticed that an increase of SNPs activity over DPPH with the increase in the SNPs concentration [55]. Hydrogen donating quality as well as aptitude of antioxidants can do DPPH free radical scavenging [6]. Antioxidants were well known as anti-mutagenic agents [56, 57].

$IC_{50}$ - the microgram of extract, SNPs to scavenge 50% of the radicals.

### *3.2. In-vitroAnti-inflammatory Assay - Inhibition of Albumin Denaturation (Dunnet Method)*

***Table 2.*** *Effect of Leaf, Bark, Fruit extracts and Leaf, Bark, Fruit SNP on heat induced protein denaturation Treatment (s) Concentration (1mM).*

| S. No. | Treatment and Concentration (1Mm) | Absorbance at 660 nm | % Inhibition of protein denaturation |
|---|---|---|---|
| 1 | Control | 0.234 ± 0.002 | - |
| 2 | Diclofenac sodium | 0.113 ± 0.006 | 51.960 ± 0.229 |
| 3 | Leaf extract | 0.197 ± 0.006 | 15.803 ± 0.245*** |
| 4 | Leaf SNP | 0.173 ± 0.0002 | 26.087 ± 0.606*** |
| 5 | Bark extract | 0.144 ± 0.006 | 38.450 ± 0.240*** |
| 6 | Bark SNP | 0.111 ± 0.001 | 52.730 ± 0.635 |
| 7 | Fruit extract | 0.214 ± 0.0009 | 08.563 ± 0.267*** |
| 8 | Fruit SNP | 0.184 ± 0.001 | 21.373 ± 0.569*** |

Note: Each value represents the mean ± SD. N=3, Experimental group were compared with control ***p considered extremely significant.

Results revealed that leaf, bark, and fruit extracts were having anti-inflammatory properties whereas these properties were increased improved in SNPs conditions. Maximum inhibition of 52.73% was observed in SNPs synthesized from Bark. SNPs synthesized from leaf were showing effect with 26.087%. SNPs synthesized from the fruit were showing effect with 21.373% which was less effective compared to SNPs from leaf and bark. All the results were compared with

Diclofenac which is a standard anti-inflammation drug that showed the maximum inhibition of 51.96% at the concentration of 1mM Table 2.

The *In-Vitro* anti-inflammatory test results of SNPs synthesized from leaf, bark, and fruits of *T. pallida* show that Inhibition of albumin denaturation Protein. Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented because of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, the ability of SNPs plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation. The results were represented in Figure 4 a bar graph it gives a clear picture of the comparison of the effectiveness of three types of SNPs synthesized. The Diclofenac was taken as a standard drug for all the extract and SNPs solutions.

In the present study, results indicate that the aqueous SNPs synthesized from leaf, bark, and fruits of *T. pallida* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenol compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. The SNPs fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat-induced albumin denaturation. This study gives on the idea that the compound of the plant *T. pallida* can be used as a lead compound for designing a potent anti-inflammatory drug that can be used for the treatment of various diseases such as cancer, neurological disorder, aging, and inflammation. As a part of testing, it was keenly observed that the inhibition of denaturation of protein by SNPs synthesized from various parts shows different values in results under the same concentrations.

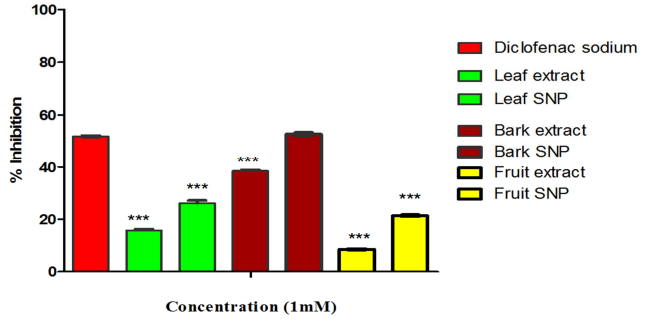


***Figure 4.*** *Effect of Leaf, Bark, and Fruit extracts and Leaf, Bark, Fruit SNP on heat-induced protein denaturation Treatment (s) Concentration (1mM). Each value represents the mean ± SD. N=3, the Experimental group was compared with control ***p considered extremely significant.*

## 4. Conclusion

The DPPH scavenging assay of SNPs synthesized by using fruit and leaves extracts of *T. pallida* showed significant scavenging activity compare with bark mediated synthesized SNPs. But the SNPs synthesized from fruit extract are showing nearly equal to standard (Ascorbic acid). The anti-inflammatory activity of SNPs synthesized from bark extract is showing higher than the standard (Diclofenac), whereas the SNPs synthesized from leaves and fruit extracts showed moderate effect. These results proved that each part has its capacity in responding to different activities, the present study regarding antioxidant and anti-inflammatory tests by synthesized silver nanoparticles from the leaf, bark, and fruit of the *Terminalia pallida* plant proved that parts of this endemic plant have remarkable potential, as reflected in the good results. In the present study we report the successful synthesis of clean, nontoxic and biocompatible SNPs using leaf, bark and fruit extract of *T. pallida.* Thus the results clearly represented the characteristic features, antioxidant and anti-inflammatory activities of SNPs.

## Declaration

### *Availability of Data and Material*

Not applicable. As a corresponding author I read and approved the final manuscript.

### *Competing Interests*

The authors declare that they have no competing interests.

### *Funding*

Not applicable.

### *Authors' Contributions*

PRM collocted plant material, and performed experiment, data analysis and wrote the manuscript. NS designed the work and finalized the manuscript.

## Acknowledgements

I'm very grateful to UGC-BSR – GATE* Fellowship for Financial assistance for this work, Department of Botany, Sri Venkateswara University, Tirupati, and I'm very grateful to research supervisor and collogues who helped me directly or indirectly to my research work.

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