In -Vitro Antioxidant and Nitric Oxide Scavenging Activity of *Shorea Robusta* Plant Leaves

[*1]Sanjay Singh and [2]Rahul Singh Dhariyal

[1] Principal Siddhartha Institute of Pharmacy, Dehradun UK., 248001,

[2] Associate Professor, Siddhartha Institute of Pharmacy, Dehradun UK., 248001

[*]Email: sanjaymph@gmail.com

DOI 10.51129/ujpah-2025-39-2(12)

Received- 20 December, 2025
Revised- 22 December, 2025
Accepted- 24 December, 2025
Published- 27 December, 2025

Abstract - This study illustrates the discussion of the activity of *Shorea Robusta* plant (known for its medicinal properties) leaves extract prepared via methanolic extraction **to assess** in-vitro antioxidant & nitric oxide scavenging activity**.** The two widely accepted methods that are- 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay along with Nitric Oxide scavenging assay compared with Ascorbic Acid **as** a standard was taken to assess the antioxidant potential. The extract evaluated at five concentrations (20, 40, 60, 80, and 100 µg/mL). In both assays, extract showed increase in concentration-dependent in radical scavenging activity**,** indicating its effectiveness in neutralizing free radicals. The DPPH assay showed a maximum inhibition of 81.76% at 100 µg/mL, in the other hand Ascorbic Acid demonstrated 95.89% inhibition at the same concentration. Similarly, in the NO scavenging assay, the extract exhibited significant inhibition, although slightly lower than the standard. The results highlight that the leaves extract possesses notable antioxidant and NO scavenging properties, which can lead to the presence of flavonoid, phenolic compounds. Although extract exhibited relatively lower activity compared to the pure standard, it still showed substantial free radical neutralizing potential, supporting its traditional medicinal use. This study that *Shorea Robusta* leaves can be a promising source of antioxidants for therapeutic applications, particularly in preventing oxidative stress-related disorders. Future research are warranted to work on the isolation of active compounds as well as investigate their mechanism of action in biological systems.

Key words: *Shorea Robusta*, Antioxidant activity, DPPH assay and Nitric oxide scavenging

Introduction

The generation of reactive oxygen species and the intracellular ability to eliminate ROS are dispro-portionate, resulting in oxidative stress. It causes significant damage to all biomolecules, including lipids, proteins, DNA, and RNA[1]**.** This damage can lead to the development of cancer, oxygen toxicity, aging, lipofuscinosis, and liver injury[2,3]**.** They may act as antioxidants against a variety of free radical-related illnesses[4]**.** Plants' anti-oxidant activity is caused by phytochemicals that interact. Antioxidant are

chemicals that inhibits oxidation or oxidative damage cause by free radical. As a result, it has the potential to neutralize reactive oxygen species and free radical. The existence of these phytochemicals in plant products has also led to recent research demonstrating that they can interact with other species in the environment to obstruct the growing of bacteria or fungi. Because these chemicals inhibit infections and have little toxicity to host cells, they are expected to provide the groundwork for the creation of novel antimicrobial drugs[5].

Plants have served as a significant source of medicinal properties for millennia. Plants are utilized for therapeutic purposes in many places and serve as the origin of numerous effective pharmaceuticals. Primarily focused on traditional medicines, particularly plants, which have a historical significance in popular folk medicine[6,7]. In Ayurveda, *Shorea robusta* is considered a significant medicinal plant. S. robusta Gaertn. f. is a member of the Dipterocarpaceae family and has historically been used to cure a variety of conditions, including wounds, ulcers, leprosy, cough, gonorrhea, earaches, headaches, and many more. Traditionally, the plant's many parts have been utilized for a variety of purposes[8,9]. The leaves have applications in Ayurveda to treat anthelmintics, inflammation, antinociception, hyperlipidemia, antioxidants, and alexiteric conditions. Wounds, leprosy, earache, ulcers, itching, gonorrhea, cough and headache are all treated with the leaves. To halt bleeding and encourage wound healing, crushed stem bark administered[10,11]. The resin is used in the Unani medical system to treat menorrhagia, spleen enlargement, and eye irritations. The resin has analgesic, antiulcer, and anti-bacterial properties. Its resin combined with sugar or honey is used for sluggish digestion, gonorrhea, and dysentery. The chopped bark releases oleoresin, which has detergent and astringent qualities[12,13]. Drops made from its bark decoction are used to treat ear issues. Additionally, diarrhea is treated using its fruits[14-17]. The primary purpose of this study was to highlight the traditional and contemporary medicinal pharmacological profile of the S. robusta plant, which is a member of the Dipterocarpaceae family and might be a source for future research.

Plant Profile [18-19]

Botanical name: Shorea. Robusta
Family: Dipterocarpaceae
Vernacular names: Guggilam, Ashvakarna, Chiraparna, Sal, Sala, Sal tree, Common Sal, Indian Da mmer, Dhuna, Damar, Jall, Sal, Salwa, and Shal
Commonly used Parts: Resin, leaves, bark, and fruit
Chemical constituents: Flavonoids, saponins, steroids, tannins, phenols, triterpenoids.

The plant's resin is fumigated in ill people's rooms and used with sugar or honey to cure bleeding piles and diarrhea. It is thought of as a detergent and astringent. It is also used to treat gonorrhea and bad digestion. Its bark extraction is used

as medicine for hearing problems, while its fruits are utilized for alleviating diarrhea. Indian Shorea robusta dammar resin. It comes out of the tree's bark fissure as a gum resin. The European Pharmacopeia states that this drug is advantageous. It comes in two varieties, white and red, is soluble in alcohol, and is used for fumigation similar to frankincense. It is combined with sulfur and applied as an ointment to wounds, etc. It is also used with wax to make plasters for wounds. It is unpleasant, strong, and bitter. It is prescribed by traditional doctors for venereal ailments such as gout and gonorrhea. It works well for bronchitis, leucorrhea, piles, and cough when combined with boiling milk. All of the system's morbid fluid can be absorbed by it. Additionally, a growing number of Hindu homes, temples, and medical facilities employ the resin[20,21].

Figure-1 Leaves part of *Shorea Robusta* plant

Material and Method

Chemicals- For the in-vitro antioxidant and nitric oxide scavenging assays of *Shorea robusta* leaves, the following chemicals and reagents were used. For DPPH radical scavenging assay- 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Methanol, Ascorbic acid remained bought by Shaila enterprise. Analytical grade reagents were utilized in all other cases. For the nitric oxide assay, **sodium nitroprusside** used in order to generate nitric oxide in solution, and **Griess reagent** was used to detect nitrite formation at **546 nm**. Extract and reagents was prepared in **methanol or phosphate buffer (pH 7.4)** as appropriate. All experiments were performed using standard laboratory glassware, pipettes, and cuvettes, and measurements were carried out in **triplicate** for accuracy. **Distilled water** was used for all dilutions.

Collection and authenticfication of Plant material- *Shorea Robusta* plant leaves has been taken from the local area of Dehradun, Uttarakhand and were air dried in the shade.

Authentication of the Plant- Authentication of *Shorea Robusta* was done by Botanical Survey of India, Dehradun, Uttarakhand.

Preparation of plant extract

Shorea robusta plant leaves were gathered, chopped into tiny pieces, shade-dried at room temperature, and then ground into a fine powder using a grinder combination. Shorea robusta powder was macerated for three days at room temperature using 70% methanol. The supernatant was moved onto a china dish after three days. By holding the china dish over a hot water bath at 45°C, the supernatant was entirely eliminated. After the alcohol was completely removed, a semi-solid extract was produced. For later usage, the resulting residue was stored in the refrigerator[22].

In-vitro Antioxidant activities

DPPH assay methods were used to determine the antioxidant activity of the plant extraction. Entirely, assay was carried out in triplicate or typical value were considering and The plant extract's capacity to scavenge nitric oxide radicals produced from sodium nitroprusside in phosphate buffer and Griess reagents was used to assess its antioxidant activity.

DPPH Radical Scavenging Assay

The capacity of Shorea Robusta leaves methanol extract to neutralize free radicals is measured. The solutions (0.004% w/v) were made using 95% ethanol. Stock solutions (10 milligrams per 100 milliliters) were made by combining the methanolic extracts of the plants with 95% ethanol and water, respectively. Two milliliters, four milliliters, six milliliters, eight milliliters, and ten milliliters of this solution in stock were added to five test tubes. & The final volume of each test tube was increased to 10 ml by successive dilutions using comparable solvents, with concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 8060 µg/ml, and 10060 µg/ml for each extract, respectively. Each of these test tubes was filled with freshly made solutions (0.004% w/v), and after ten minutes, the absorbance was measured at 517 nm using a spectrophotometer. In order to create stock solutions with comparable quantities, ascorbic acid was either dissolved in distilled water or used as a reference standard medication. The same quantities were present in a control sample that was prepared. The following formulas were used to calculate the percentage of free radical scavenging. [328-330].

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

(A absorbance of control)

Nitric Oxide Scavenging Assay

At dosages of 20, 40, 60, 80, and 100 µg/ml, the nitric oxide scavenging ability of its leaf extract was evaluated using ascorbic acid as a reference. Sodium nitroprusside (5 mM) was mixed with varying amounts of the extract or standard in phosphate buffer (pH 7.4) and left to rest at room temperature for 150 minutes. After incubation, an equal quantity of Griess reagent was added to each mixture to interact with the nitrite that nitric oxide had created. Using a UV-visible spectrophoto-meter, the absorbance of the

resulting pink solution at 546 nm was measured. The percentage suppression of nitric oxide radicals was determined by comparing the test samples' absorption coefficient with the extract-free control solution.

% 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

Table-1 Preliminary phytochemical investigation of *Shorea Robusta*

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

Table I: + represents presence; ++ represents present in more concentrations; - represents absence. Thephytochemical testing of methanolic extraction of leave of *Shorea Robusta* shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

Phytochemical screening- *Shorea Robusta* leaves extraction on screening shows plants is riches in various actives ingredient (2^{nd}ry plant metabolite). The results of phytochemicals screening exposed strongest to moderates occurrence of alkaloid, flavonoid, saponins and carbohydrates (Table-1).

In-Vitro Antioxidant Activity

DPPH radical scavenging activity

Shorea Robusta leaves extract at two different concentrations investigate in this present study was examine along with specific antioxidants (Ascorbic Acid) at their similar concentration. *Shorea Robusta*

leaves extract (methanolic extracts) presented significant effects in concentration dependents manner. The extracts exhibit comparatively lower DPPH free radical scavenging potentials when compared to the conventional antioxidant applications in the trials.

Table-2 *Shorea Robusta* leaves extract antioxidant activity by DPPH method.

S.NO.	Methanolic Leaves Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	DPPH % Percentage Inhibition	Concentration (µg/mL)	Percentage Inhibition
01	20	22.12	20	35.23
02	40	38.89	40	55.16
03	60	52.45	60	70.45
04	80	68.23	80	85.56
05	100	81.76	100	95.89

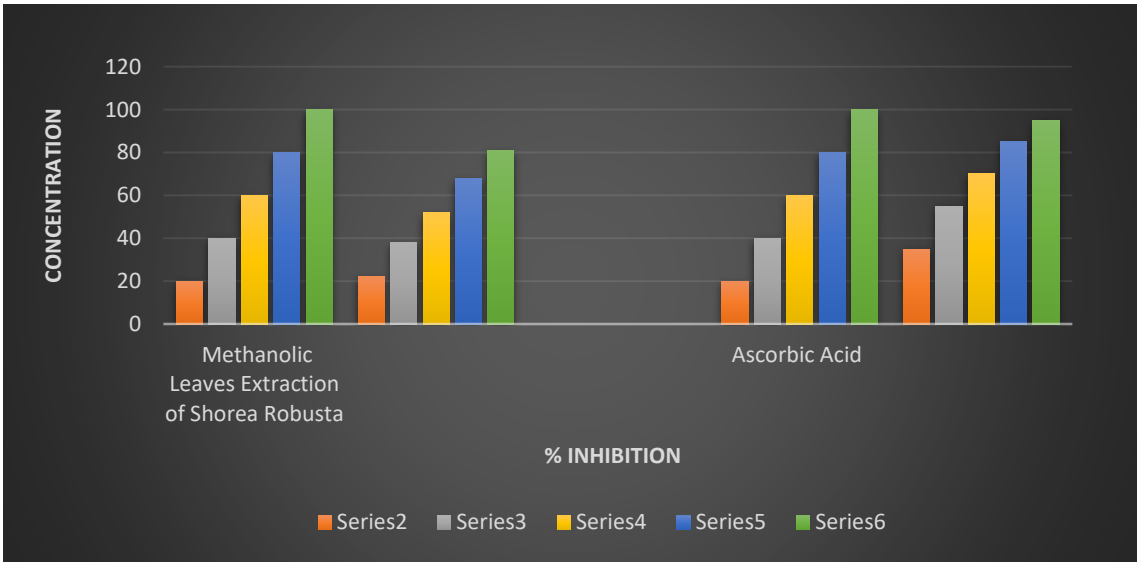

Figure-1 Graphical representation I- shows the % inhibition of DPPH radicals by extraction of *Shorea Robusta*

Table - 3 Antioxidant activities of methanolic extraction of leaves part of *Shorea Robusta* by DPPH method.

S. NO.	Methanolic Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	Absorbance at 517nm	Concentration (µg/mL)	Absorbance at 517 nm
01	20	0.624	20	0.520
02	40	0.496	40	0.360
03	60	0.384	60	0.240
04	80	0.256	80	0.120
05	100	0.152	100	0.040

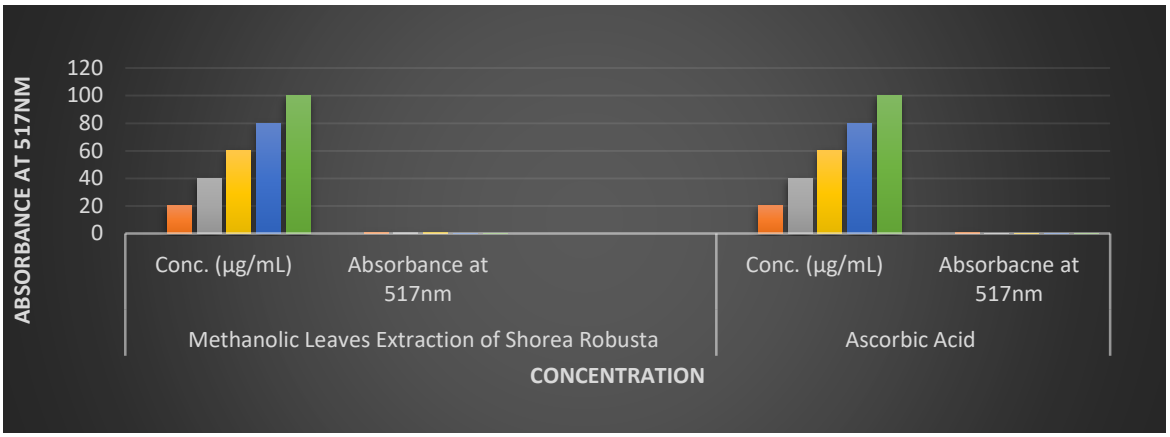

Figure-2 Graphical representation II showing the absorbance of DPPH radicals by extracts of *Shorea Robusta*

Scavenging Activity of Nitric Oxide

The nitric oxide radical scavenging strength of Shorea robusta leaf extract at various doses explored in the current study was compared with Ascorbic Acid at the same concentrations. Shorea robusta leaf extract (methanolic extract) demonstrated considerable nitric oxide radical scavenging activity in a concentration-dependent manner. When compared to the conventional antioxidant employed in the trials, the extract demonstrated much lower nitric oxide radical scavenging capability.

Table -4 Antioxidant activities of methanolic extraction of leaves part of *Shorea Robusta* by Nitric Oxide (NO) Scavenging method.

S.NO.	Methanolic Leaves Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	Nitric Oxide % Inhibition	Concentration (µg/mL)	Percentage Inhibition
01	20	18.12	20	30.34
02	40	34.76	40	50.76
03	60	49.87	60	65.98
04	80	63.32	80	78.14
05	100	77.0	100	88.76

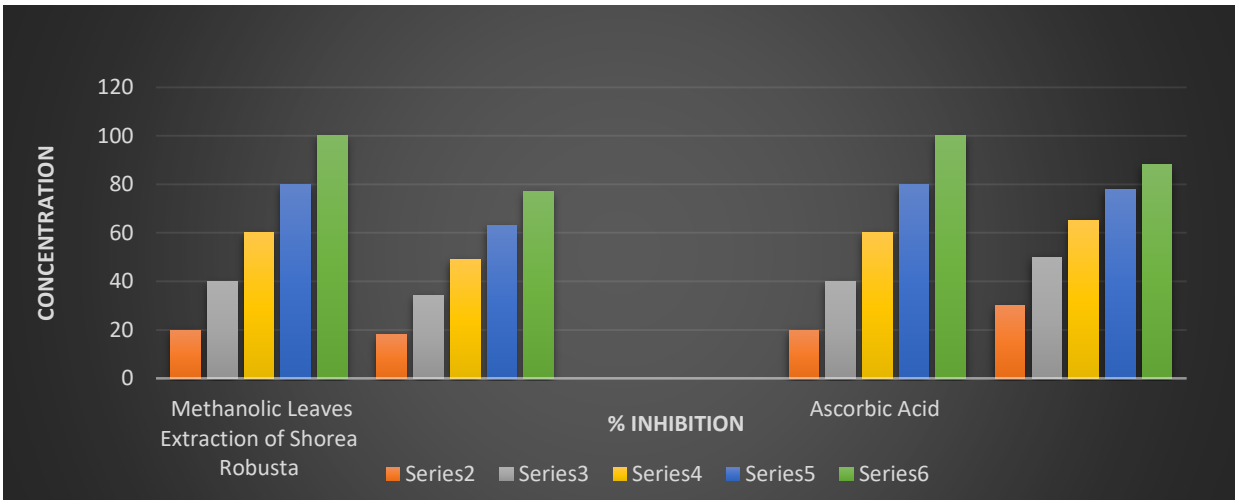

Figure-3 Graphical representation III shows the % inhibition of Nitric oxide radicals by extraction of *Shorea Robusta*

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

S.NO.	Methanolic Leaves Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	Nitric Oxide Absorbance at (546nm)	Concentration (µg/mL)	Absorbance at (546nm)
01	20	0.656	20	0.560
02	40	0.528	40	0.400
03	60	0.408	60	0.280
04	80	0.296	80	0.176
05	100	0.184	100	0.096

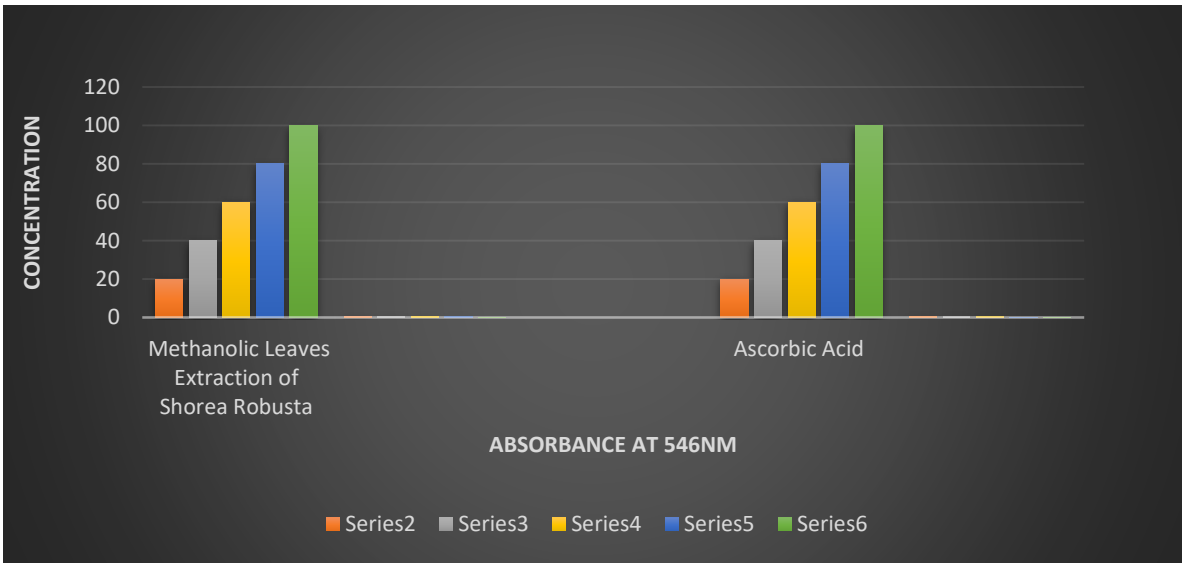

Figure-4 Graphical representation IV shows the % inhibition of Nitric oxide radicals by extraction of *Shorea Robusta*

Discussion

The current study used both DPPH and nitric oxide scavenging tests to assess the in vitro antioxidant activity of Shorea robusta methanolic leaf extract. The findings showed that the extract significantly scavenged radicals in both tests in a concentration-dependent manner. This indicates that higher concentrations were showing good results in neutralizing free radicals when compared to standard. Ascorbic Acid, the *Shorea robusta* extract showed relatively lower % inhibition at all tested concentrations. This is consistent with the observation that natural plant extracts often contain a mixture of bioactive compounds, which may act synergistically but are generally less potent than pure standard antioxidants in vitro. Nevertheless, the extract still showed **substantial scavenging activity**, confirming its potential as a natural source of antioxidant compounds. The DPPH assay, which measures extract's capacity to give hydrogen to stabilize DPPH radicals, showed a maximum inhibition of approximately 81.76% at 100 μg/mL, while Ascorbic Acid showed 95.89% inhibition at the same concentration. Similarly, in the Nitric Oxide scavenging assay, the extract demonstrated effective inhibition of NO radicals, which are implicated in oxidative stress and inflammation. The activity of the extract in the NO assay was slightly lower than in the DPPH assay, suggesting that the extract may have **more pronounced hydrogen-donating antioxidant activity** than NO scavenging potential. These results align with previous studies on plant antioxidants, where methanolic extracts of leaves often contain **phenolic and flavonoid compounds**. The concentration-dependent increase in activity indicates that *Shorea robusta* leaves are a promising candidate for **natural antioxidant development**. Overall, the comparative analysis with Ascorbic Acid confirms that while the extract is less potent than the standard, it still exhibits significant antioxidant and nitric oxide scavenging potential, which may justify its traditional use in herbal remedies and supports further investigation for therapeutic applications.

Conclusion

The current investigation also showed that *Shorea Robusta* leaves portions includes a range of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may having enormous potential uses as a remedy for a variety of long-term conditions. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence. More research is required to produce innovative antioxidant medications.

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 composition and in vitro antioxidant evaluation 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, J. Pharmacognosy, Phyto-chemistry of Medicinal Plants. *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, EM. Documenting and evaluating herbal remedies. *Econ. Bot.;* 1983; 37:13–27.

8. Ministry of Health, Government of India; New Delhi: Govt. of India. *The Ayurvedic Pharmacopoeia of India;* 2001.

9. Sharma, P.C.; Yelne, M.B. and Dennis, T.J. *Database on Medicinal Plants Used in Ayurveda.* 3rd ed. New Delhi: *Central Council for Research in Ayurveda and Siddha*; 2005; $p_{p.}$ 282–4.

10. Chandel, K.P.; Shukla, G. and Sharma N. *Biodiversity in Medicinal and Aromatic Plants in India: Conservation and Utilization.* New Delhi: National *Bureau of Plant Genetic Resources*; 1996.

11. Chitale, V. S. and Behra, M.D. Can the distribution of Sal (*Shorea robusta* Gaertn.f.) shift in the northeastern direction in India due to changing climate? *Curr Sci.;* 2012; 102: 1126–35.

12. Patwardhan, B.; Warude, D.; Pushp-angadan; P. and Bhatt, N. Ayurveda and traditional Chinese medicine: A comparative overview. *Evid Based Complement Alternat. Med.;* 2005; 2:465–73.

13. Misra, B. and Vaisya, R. *Tailavarga in Bhavaprakasha Nighantu.* Part I(B). Varanasi: Chaukhumba Bharati Academy; 1963. $p_{p.}$ 779.

14. Patwardhan, B.; Chopra, A. and Vaidya, A. D. Herbal remedies and

the bias against Ayurveda. *Curr Sci.;* 2003; 84:1165–6.

15. Vaidya, R.A.; Vaidya, A.D.; Patwar-dhan, B.; Tillu, G. and Rao, Y. Ayurvedic pharmaco-epidemiology: A proposed new discipline. *J. Assoc. Physicians India;* 2003; 51:528.

16. Bursal, E. and Gulçin, I. Polyphenol contents and in vitro antioxidant activities of lyophilized aqueous extract of kiwifruit (*Actinidia deliciosa*). *Food Res Int.;* 2011; 44: 1482–9.

17. Morikawa, T.; Chaipech, S.; Matsuda, H.; Hamao, M.; Umeda, Y. and Sato, H. et al. Antidiabetogenic oligostilbenoids and 3-ethyl-4-phenyl-3,4-dihydro-isocoumarins from the bark of *Shorea roxburghii*. *Bioorg Med Chem.;* 2012; 20: 832–40.

18. Kokate, C.K. and Purohit, A.P. *Pharmacognosy.* 38th ed.; Pune: Nirali Prakashan; 2005; pp. 600–4.

19. Kar, A. *Pharmacognosy and Biotechnology.* 2nd ed.; New Delhi: New Age International Pvt. Ltd.; 2007.

20. Chunekar, K. C. and Pandey, G. S. *Bhavaprakasha Nighantu.* Varanasi: Chaukhambha Bharati Academy; 2010; pp. 508–9.

21. Sastry, J.L. *Dravyaguna Vijnana.* Varanasi: Chaukhambha Orientalia; 2005; pp. 78–6.

22. Suganya, P.; Nandhini, R.; Jeyadoss, T. and Velavan, S. In vitro antioxidant activity of methanolic extract of *Shorea robusta* in hepatocytes. *Int. J. Pharm. Sci.;* 2014; 6(6): 227–30.