



## RESEARCH ARTICLE

# Antioxidant profiling of *Garcinia talbotii* Raizada ex. Santapau

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## Abstract

The genus *Garcinia* was originally classified under the family *Guttiferae* Juss. according to Bentham and Hooker's system of classification. However, it has now been reclassified under the family *Clusiaceae* Lindl. according to the APG III system of classification. It is represented by over 400 species; 35 species are found in India and the rest are distributed in the tropics of the world. The *Garcinia talbotii* Raizada ex. Santapau is native and common in dry plains. It thrives in a monsoon climate characterised by a distinct dry season. The tree grows up to an elevation of 100-500 m in the Western Ghats. This plant genus has been used medicinally since ancient times. Traditional herbal treatments are becoming increasingly popular owing to their ease of use, low cost, and absence of side effects. Antioxidants are compounds that can neutralize harmful free radicals in the body, which can damage cells and contribute to various health issues, including cancer, cardiovascular diseases, and aging. Plants can deliver a huge number of differing bioactive compounds which may supplement the requirements of the human body by acting as natural antioxidants. The present study explores the antioxidant activity of *Garcinia talbotii* leaves, utilizing both aqueous and hydroalcoholic extracts for analysis. DPPH:2,2-diphenyl-1-pirylhydrazyl free radicals scavenging assay, FRAP: Ferric Reducing Antioxidant Power Assay, Nitric oxide free radical scavenging activity, Phosphomolybdate and Hydrogen peroxide method were used to screen the antioxidant activity of the extracts and ascorbic acid was used as positive control. In the FRAP assay, the aqueous extract showed the highest antioxidant activity  $r^2 = 0.9062$  compared to the standard and hydroalcoholic extract. In the hydrogen peroxide assay, the aqueous extract exhibited the highest antioxidant activity  $r^2 = 0.9944$  compared to both. In the nitric oxide assay, the aqueous extract again showed the highest antioxidant activity  $r^2 = 0.9859$ . In the phosphomolybdenum assay, the aqueous extract showed the highest activity  $r^2 = 0.9966$ , followed by the hydroalcoholic extract  $r^2 = 0.9438$  and the standard  $r^2 = 0.9326$ . However, in the DPPH assay, the standard exhibited the highest antioxidant activity  $r^2 = 0.9870$ . The aqueous and hydroalcoholic extracts showed significantly higher antioxidant activity compared to the standard.

**Keywords:** *Guttiferae* Juss., *Clusiaceae* Lindl. DPPH:2,2-diphenyl-1-pirylhydrazyl free radicals scavenging assay, FRAP: Ferric Reducing Antioxidant Power Assay, Nitric oxide, Phosphomolybdate, Hydrogen peroxide, Ascorbic acid

## Introduction

Ayurveda, the oldest medical system still in use in India, is founded on a strong experimental and philosophical basis. It is a life science that emphasises personalised care

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and offers a holistic perspective on wellness. Recognized as a comprehensive medical system, Ayurveda considers the physical, psychological, ethical, philosophical, and spiritual aspects of a person's health. Often referred to as the science of self-healing, it views every cell as a reflection of pure intelligence. In this ancient Indian system, self-healing is considered just as vital as the use of herbal remedies (Balachandran and Govindarajan 2005).

The Indian subcontinent is rich in medicinal herbs, many of which are integral to traditional medicine. For a long time, Indian medical systems have been regarded as invaluable sources of knowledge by Western societies. Of the over 20,000 medicinal herbs identified in India, only 7,000-7,500 are currently used by indigenous tribes to treat various ailments. Today, most medications are still derived from plants, animals, minerals, and metals. The production of Ayurvedic medicines by major pharmaceutical companies continues to rely heavily on plant products (Ballabh *et al.* 2007). The rising popularity of traditional herbal remedies

can be attributed to their affordability, ease of use, and lack of side effects.

Antioxidants are a group of naturally occurring compounds that help reduce or neutralise oxidative stress within the body's physiological systems. They are commonly found in various foods. The body constantly produces free radicals because it uses oxygen regularly. These free radicals harm bodily cells and have been connected to a variety of diseases, such as diabetes, cancer, heart disease, and macular degeneration. Antioxidants are great at scavenging free radicals, which helps to both prevent and repair the damage these radicals cause to cells. Antioxidants that occur naturally are widely distributed in both plants and animals. Other techniques for synthesising antioxidants from different agricultural wastes include chemical and biological synthesis. Based on their solubility, antioxidants can be broadly classified as either lipid soluble or water soluble. Antioxidants that are soluble in water, such as glutathione, uric acid, and ascorbic acid, often function within the cytosol and blood plasma of cells. Lipid-soluble antioxidants like ubiquinol, α tocopherol, and carotenoid protect cell membranes from lipid peroxidation, while ascorbic acid acts as a redox catalyst, reducing and neutralising reactive oxygen species (ROS). Glutathione, on the other hand, is a reducing agent with antioxidant properties that can be oxidized and reduced reversibly. Based on how they function, two more often employed groups of antioxidants are primary or chain-breaking antioxidants and secondary or preventative antioxidants. Antioxidants can also act as prooxidants when they are lacking at the incorrect time, location, or concentration (Dontha 2016, Krishnaiah *et al.* 2011).

The genus *Garcinia* was originally classified under the family Guttiferae Juss. according to Bentham and Hooker's classification system. However, advancements in phylogenetic research led to its reclassification under the family Clusiaceae Lindl. in the APG III system. This revision provides a more precise understanding of the evolutionary relationships within these plant groups (Angiosperm Phylogeny Group., 2009)

Globally, *Garcinia* encompasses over 400 species, primarily distributed across tropical regions. In India, around 35 species have been recorded, with many being endemic to the Western Ghats and northeastern states (Singh D & Singh RK 2014, Sabu M & Shareef S 2021). Prominent species such as *Garcinia indica* (kokum), *Garcinia gummi-gutta* (Malabar tamarind), and *Garcinia xanthochymus* (yellow mangosteen) play vital roles in local ecosystems. Their significant economic value stems from their extensive use in culinary, pharmaceutical, and industrial applications (Deb P & Parimoo D 2015, Poovachal 2020, India Biodiversity Portal)

*Garcinia talbotii* was first described by H. Santapau in 1960, building upon the work of A.R.K. Raizada. It was initially documented in the Gairsoppah Ghats of North Kanara, Karnataka, India. The species is named in honor

of botanist C.R. Talbot, acknowledging his significant contributions to the study of Indian flora (Santapau H 1960, Raizada ARK 1958). *Garcinia talbotii* Raizada ex. Santapau is a medium-sized tree native to the Western Ghats. Its branches are angular and striate, with coriaceous leaves measuring 5-10 cm in length and 4-6 cm in width. The leaves are elliptic-oblong, obtuse or retuse, and rarely sub-acute, with prominent reticulate veins visible in dried specimens. The petioles are 1 cm long, stout, and transversely rugose (Almeida 1996). The tree is known to flower between March and May, producing fruits with a sweet pulp, similar to other species of *Garcinia* (Almeida 1996, Jain *et al.* 2023).

*Garcinia talbotii* possesses a diverse array of bioactive compounds that contribute to its medicinal properties. Phytochemical analyses have identified the presence of alkaloids, phenols, terpenoids, tannins, fats, steroids, saponins, glycosides, gums, mucilage, and fixed oils in different parts of the plant. Notably, its roots contain two biflavones, talbotaflavone and morelloflavone, which may play a key role in its pharmacological potential (Patil SB, Wadd NV, 2022). Furthermore, research indicates that the bark extract of *Garcinia talbotii* possesses antioxidant and anti-inflammatory properties, largely due to its high concentration of phenolic compounds and xanthones (Jain *et al.* 2023).

The current study aims to assess the antioxidant and free radical scavenging properties of *Garcinia talbotii* Raizada ex Santapau leaves.

## Material and Method

### Collection and Authentication of Plant material

*Garcinia talbotii* Raizada ex. Santapau leaves were collected from Matheran, District Raigad in Maharashtra and authenticated from the Blatter Herbarium, St. Xavier's College, Mumbai (Specimen no.: - NI-2620).

### Preparation of Extract

The leaves were cleaned, allowed to dry in the sun, and then blended into a fine powder. The aqueous and hydroalcoholic leaf extracts were prepared using soxhlet apparatus.

### Antioxidant assay

The antioxidant activity of *Garcinia talbotii* Raizada ex Santapau leaf extract in neutralizing DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was assessed using the standard protocol outlined by Shimada *et al.* (1992). The reducing power of the hydroalcoholic and aqueous extracts was measured following the procedure described by Oyaizu M (1986). The hydrogen peroxide scavenging activity of the extracts was assessed using the method described by Rush *et al.* (1989). Nitric oxide radical scavenging activity was assessed following the method outlined by Marcocci *et al.* (1994). The total antioxidant capacity was evaluated using the phosphomolybdenum assay described by Jan *et al.* (2013).

## Results

### Reducing power assay (FRAP)

In comparison to ascorbic acid (AA), Figure 1 illustrates the reductive potential of aqueous (D/W) and hydroalcoholic (HA) plant extracts. The ferric ion reducing antioxidant power activity indicates that the reducing power appears to grow with concentration. In this the hydroalcoholic extract and aqueous extract shows slightly higher antioxidant activity as compared to standard.

Superoxide scavenging activity by alkaline DMSO method/hydrogen peroxide method

The hydroalcoholic extract exhibited a concentration-dependent scavenging activity against hydrogen peroxide, with its effectiveness increasing as the concentration rose. Figure 2 illustrates that both the hydroalcoholic and aqueous extracts showed hydrogen peroxide scavenging activity similar to that of the standard, ascorbic acid. This demonstrates that both extracts are effective in neutralizing hydrogen peroxide, a key reactive oxygen species, and perform on par with the standard antioxidant.

### Nitric oxide scavenging activity

Figure 3 presents the nitric oxide scavenging activity of the aqueous (D/W) and hydroalcoholic (HA) plant extracts in comparison to the standard antioxidant, ascorbic acid (AA). The results indicate a distinct hierarchy in scavenging effectiveness, with the aqueous extract demonstrating the highest activity, followed by the hydroalcoholic extract, which in turn surpasses the standard ascorbic acid. This suggests that the aqueous extract has a superior capacity for neutralizing nitric oxide radicals, while the hydroalcoholic extract also exhibits significant activity, outperforming ascorbic acid.

### Phosphomolybdenum assay

The total antioxidant activity in the phosphomolybdenum assay increases progressively with rising concentrations of the extracts as shown in Figure 4. The aqueous extract displayed a strong linear relationship between concentration and antioxidant activity, with a coefficient of correlation ( $r^2$ ) of 0.99, indicating near-perfect consistency. In contrast, the hydroalcoholic extract exhibited antioxidant activity comparable to that of the standard, ascorbic acid, across the tested concentrations. These findings suggest that while both extracts demonstrate significant antioxidant potential, the aqueous extract shows a more direct concentration-dependent increase in activity.

### DPPH Free radical scavenging activity

The DPPH radical scavenging activity of the hydroalcoholic (HA) and aqueous (D/W) extracts was compared with ascorbic acid (AA), which served as the standard antioxidant, as illustrated in Figure 5. This comparison emphasizes the antioxidant potential of both *Garcinia talbotii* extracts

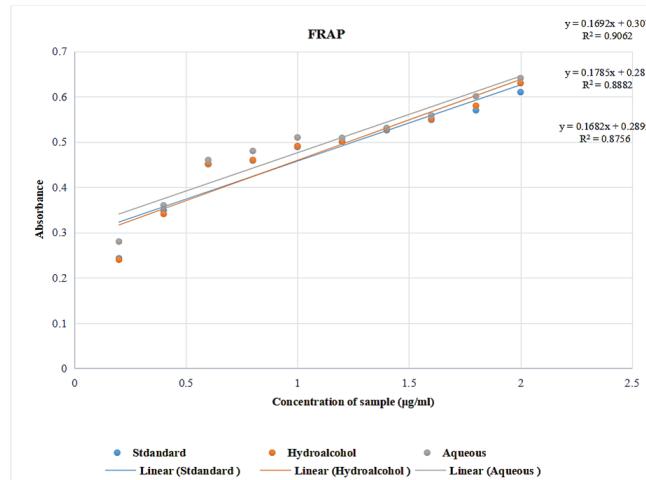


Figure 1: Reducing power assay (FRAP) of *Garcinia talbotii* Raizada Ex. Santapau leaves -hydroalcoholic extract vs. aqueous vs. standard

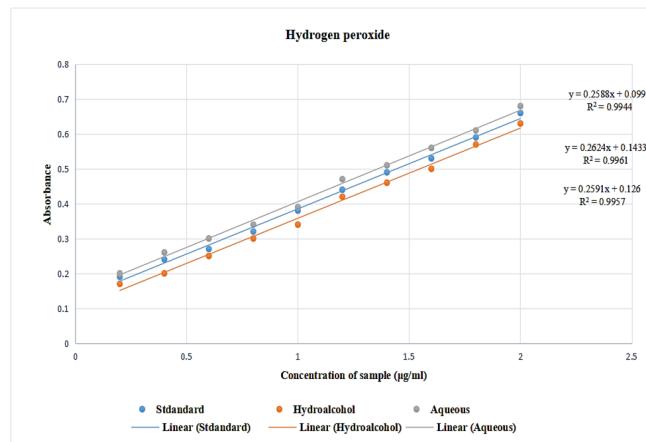


Figure 2: Superoxide scavenging activity by alkaline DMSO method/ hydrogen peroxide method of *Garcinia talbotii* Raizada Ex. Santapau leaves hydroalcoholic extract vs. Aqueous vs. Standard

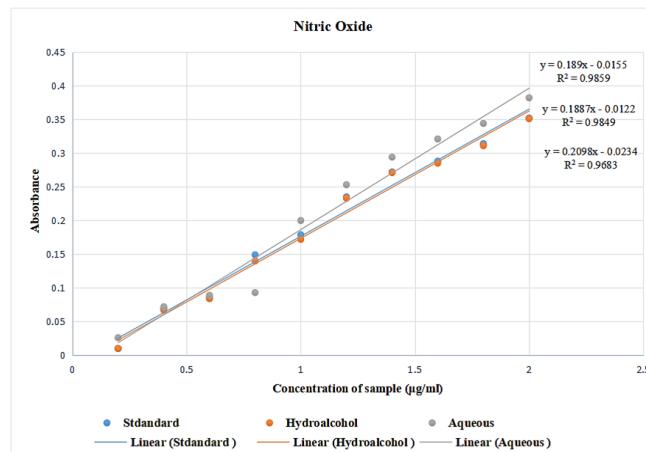


Figure 3: Nitric oxide scavenging activity of *Garcinia talbotii* Raizada Ex. Santapau leaves hydroalcoholic extract vs. aqueous vs. standard

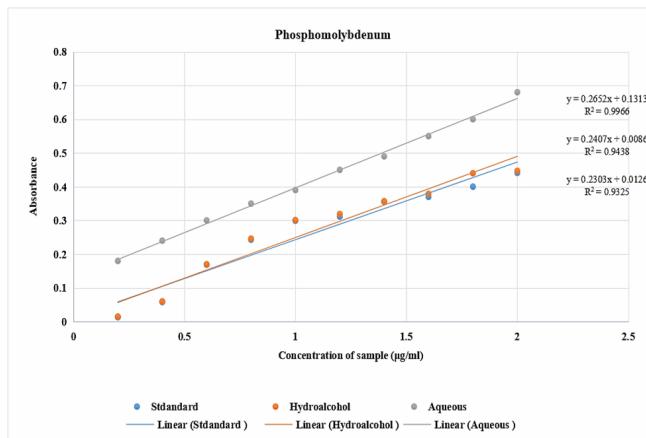


Figure 4: Phosphomolybdenum assay of *Garcinia talbotii* Raizada Ex. Santapau leaves hydroalcoholic extract vs. aqueous vs. standard

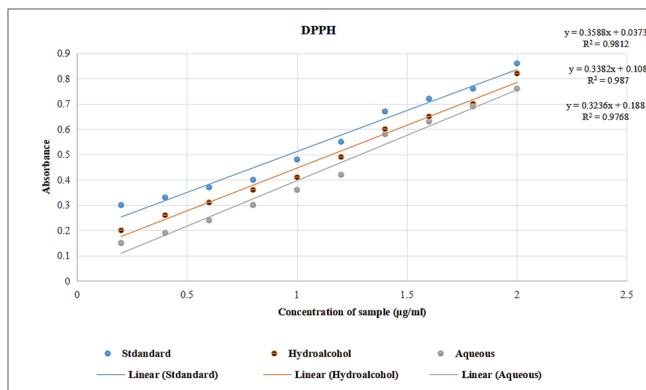


Figure 5: DPPH free radical scavenging activity of *Garcinia talbotii* Raizada Ex. Santapau leaves hydroalcoholic extract vs. aqueous vs. standard

relative to ascorbic acid. Notably, the results indicate that both the hydroalcoholic and aqueous extracts exhibited lower DPPH scavenging activity to that of the standard, ascorbic acid. This suggests that the extracts possess a strong capacity to neutralize free radicals, making them comparable in efficacy to a well-established antioxidant like ascorbic acid.

## Discussion

Plant-based antioxidants appear to have a bright future as research on these substances progresses. It is anticipated that their incorporation into skincare products, nutraceuticals, and functional meals will increase, providing customers with all-natural means of promoting wellness and averting illness. Furthermore, it is anticipated that additional research in clinical settings will confirm their therapeutic potential, making plant-based antioxidants a primary target for the creation of next-generation health-promoting goods (Lobo *et al.* 2010).

A free radical molecule called DPPH is frequently used to evaluate a sample's capacity to scavenge free radicals.

This technique was developed by Blois (1958), who also showed that a stable free radical DPPH radical could receive a H atom for the first time. This assay has garnered interest for the characterization of antioxidant activities after around thirty years (Krishnan *et al.* 2012). DPPH, a stable free radical, can accept an electron or hydrogen radical to form a stable diamagnetic molecule. Its strong absorption at 517nm makes it useful for testing plant extracts' ability to act as free radical scavengers. Antioxidants can quench DPPH free radicals, converting them to a colorless product. This technique assesses an antioxidant's capacity to lower ferric iron. Its foundation is the reduction of the ferrous form to the low pH complex of 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) containing ferric iron. Using a diode-array spectrophotometer, the decrease in absorption at 593 nm is used to measure this reduction (Antovich *et al.* 2002).

Hydrogen peroxide, though not highly reactive on its own, can generate hydroxyl radicals in cells, making it potentially toxic. It is a short-lived, reactive oxygen species that is challenging to assess. Most chemiluminescence assays for antioxidant activity use direct chemiluminescence and typically include a chemiluminescent agent, hydrogen peroxide (as an oxidant), either with or without a metal or enzymatic catalyst, and an antioxidant or extract. The ability of extracts to scavenge H<sub>2</sub>O<sub>2</sub> may be linked to their phenolic compounds, which can donate electrons, converting H<sub>2</sub>O<sub>2</sub> into water. The hydroalcoholic extract demonstrated concentration-dependent scavenging activity against hydrogen peroxide (Fereidoon *et al.* 2015).

In biological systems, nitric oxide (NO) is a diatomic free radical that is extremely reactive and has a short lifespan. NO levels can be indirectly assessed by detecting its stable metabolites, nitrate (NO<sub>3</sub>) and nitrite (NO<sub>2</sub>), through spectrophotometry. This method first converts nitrate to nitrite, followed by the Griess reaction to quantify nitrite. The Griess reaction involves two steps: sulfanilamide reacts with N<sub>2</sub>O<sub>3</sub>, forming a diazonium ion. This ion then couples with N-(1-naphthyl) ethylenediamine, resulting in the formation of a coloured azo compound that absorbs strongly at 540 nm. The enzymatic conversion of NO<sub>3</sub> to NO<sub>2</sub>, utilizing commercially available nitrate reductase, has been found to effectively quantify these molecules in extracellular fluid samples. Nitric oxide (NO) generated from sodium nitroprusside (SNP) was measured using the method described by (Marcocci *et al.* 1994).

The total antioxidant capacity assay evaluates antioxidant potential through the formation of a phosphomolybdenum complex. This assay relies on the reduction of Mo (VI) to Mo (V) by the sample analyte, resulting in the production of a green phosphate Mo (V) complex under acidic conditions (Mbinda and Musangi 2019, Munteanu and Apetrei 2021).

In the present study, the antioxidant activity of *Garcinia*

*talbotii* Raizada ex Santapau leaves were evaluated using different assays, with both hydroalcoholic and aqueous extracts showing promising results. In the DPPH, FRAP, and nitric oxide assays, both extracts demonstrated significant antioxidant activity compared to the standard, indicating their potential in scavenging free radicals. Interestingly, in the hydrogen peroxide assay, the extracts exhibited similar activity to the standard, suggesting comparable efficacy in reducing oxidative stress in this context. Furthermore, the phosphomolybdenum assay revealed that the aqueous extract exhibited higher antioxidant activity than the standard, while the hydroalcoholic extract produced comparable results. These findings highlight the differential activity of the extracts depending on the assay, underscoring the versatility of *Garcinia talbotii* leaves as a source of natural antioxidants and suggesting their potential application in pharmaceutical or nutraceutical formulations.

## Conclusion

The antioxidant activity of *Garcinia talbotii* leaf extracts (hydroalcoholic and aqueous) were evaluated using FRAP, nitric oxide, hydrogen peroxide, phosphomolybdenum, and DPPH assays, with ascorbic acid as the standard. Both extracts showed antioxidant activity comparable to or higher than the standard in most assays, except in DPPH, where the standard was superior. The aqueous extract consistently outperformed the hydroalcoholic extract, especially in the hydrogen peroxide, nitric oxide, and phosphomolybdenum assays.

All five methods proved effective and reliable for antioxidant evaluation, each offering unique insights. The phosphomolybdenum assay demonstrated outstanding efficiency and precision, making it suitable for both preliminary screenings and large-scale studies.

Collectively, these assays provide a strong foundation for advancing antioxidant research, highlighting their utility in both basic and applied studies.

To further explore the potential of *Garcinia talbotii* Raizada ex Santapau leaves, future research could focus on several key areas. Investigating the integration of these extracts into pharmaceutical products could leverage their antioxidant properties for practical applications. Additionally, evaluating their antioxidant effects in biological systems would help confirm their efficacy and potential health benefits. By addressing these areas, future research can deepen the understanding and utilization of *Garcinia talbotii* Raizada ex Santapau as a valuable natural resource for antioxidant activity.

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