Antioxidant activity of *Mitragyna parvifolia* (Roxb.) Korth

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Abstract
Antioxidants play a crucial role in protecting living organisms against oxidative stress, a condition associated with various chronic diseases and aging. Extensive research has been conducted to identify and to characterize natural compounds with potent antioxidant activity, aiming to harness their potential benefits for human health. Mitragyna parvifolia (Roxb.) Korth, a member of the Rubiaceae family, has gained considerable importance in the field of traditional medicine, particularly in the system of Ayurveda. This plant has been extensively utilized for various purposes, solidifying its significance in traditional medicinal practices.

This research aims to investigate the antioxidant activity of Mitragyna parvifolia using different methods. The plant extract was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, nitric oxide free radical scavenging activity assay and phosphomolybdate assay. The results provide valuable insights into the potential applications in the development of natural antioxidants from Mitragyna parvifolia.

Keywords: *Mitragyna parvifolia*, Rubiaceae, Antioxidant activity, DPPH, FRAP, Nitic oxide, Phosphomolybdate.

Introduction

Our bodies engage in various natural biological processes like breathing, digesting food and converting fats into energy. However, these processes also generate harmful substances known as free radicals. Normally, our body's natural antioxidant system eliminates these free radicals. But when this system fails to function properly, free radicals can initiate harmful reactions within the body. This reaction has the potential to damage cell membranes, to hinder the activity of crucial enzymes, to disrupt essential cellular processes, to impede normal cell division, harm DNA and to obstruct energy production[17]. Free radicals have a vital role in numerous biological processes, some of which are crucial for sustaining life.

Free radicals, they play a role in the intracellular eradication of bacteria by phagocytes, notably granulocytes and macrophages. Additionally, researchers have identified the involvement of free radicals in specific cellular signaling processes, commonly referred to as redox signaling[5]. The interaction between free radicals and DNA is believed to contribute to the development of numerous cancers. This interaction can cause mutations that disrupt the normal cell cycle, ultimately leading to the formation of neoplasms[23]. Free radicals and reactive oxygen species are integral components of life, engaging in crucial biological reactions[25]. The presence of antioxidant compounds is necessary to neutralize the harmful impacts of reactive oxygen species[24].

Chronic diseases such as cancers, arthritis, cardiovascular disease and vascular disorders are primarily impacted by oxidative stress. Plants, with their diverse range of antioxidant compounds, serve as a valuable reservoir of active biological substances[25]. Antioxidants play a protective role against oxidants by either eliminating free radicals or inhibiting their formation thus safeguarding cells from damage[6]. Phenolic compounds exhibit antioxidant activity by effectively neutralizing free radicals[25]. Plant phenolic compounds perform a variety of functions, such as having antioxidant properties, exhibiting antibacterial and natural pesticide properties, functioning as plant protection agents against UV waves and serving as insulating agents for plant cell walls against gases[21].

Phenolic compounds are known as excellent sources of natural antioxidants within plants. Among them, flavonoids represent the largest group of plant phenols, accounting for over half of the approximately 8,000 natural phenolic compounds[20]. The antioxidant potency of flavonoid compounds is directly linked to the increase in the number of hydroxyl groups they possess [16]. Plants have historically served as a valuable source of exogenous antioxidants for dietary purposes. It is estimated that approximately two-thirds of the world's plant species hold medicinal significance, with nearly all of them possessing remarkable antioxidant potential[12]. Medicinal plants, also known as medicinal herbs, have been utilized in traditional medicine for centuries. These plants produce a wide array of chemical compounds that serve various purposes, such as defense against insects, fungi and diseases[3].

Rubiaceae, a plant family of significant medicinal and economic importance, is found across various regions. The plant members belonging to this family holds immense medicinal value due to the diverse range of phytochemicals present within them[4]. *Mitragyna parvifolia*, commonly known as Kadamb and belonging to the Rubiaceae family, is a tree that holds substantial cultural, health and economic significance[8]. It is found in deciduous and evergreen forests

across India, contains various chemical constituents, including pyroligneous acid, methyl acetate, ketones and aldehydes. Because of its numerous medicinal properties, the plant is extensively utilized by tribal communities and Ayurvedic practitioners[13].

Extensive scientific research has established that this medicinal plant exhibits a wide range of immunopharmacological properties including analgesic, anti-pyretic, anti-inflammatory, anti-arthritic, anti-helminthic and antioxidant effects[7]. The bark and roots of the plant are utilized in the treatment of various conditions such as fever, colic, muscular pain, burning sensation, poisoning, gynecological disorders, cough and edema. Additionally, fruit juice is known to enhance breast milk production in lactating mothers[15]. The objective of this study is to assess the antioxidant activity of leaves of *Mitragyna parvifolia* by employing multiple assay methods.

Material and Methods

Mitragyna parvifolia leaves were collected from Vile parle and authenticated at Blatter Herbarium, St. Xaviers College, Mumbai (Accession no. 433). The leaves were washed, air dried and ground into a fine powder using blender. The aqueous and hydro alcoholic leaf extract was prepared using Soxhlet apparatus. The scavenging ability of *Mitragyna parvifolia* leaf extract against DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was evaluated using a standard method with appropriate modifications according to the method of Kaskoos[11]. The reducing power of *Mitragyna parvifolia* leaf extract was determined according to the method of Narayanan et al[18] whereas nitric oxide free radical scavenging activity was determined according to the method of Olatidoye et al[19]. The total antioxidant capacity assay was conducted using the phosphomolybdenum method[9].

Results and Discussion

Free radical scavenging activity: DPPH is a free radical compound commonly employed to assess the free radical-scavenging potential of various samples[10]. This method involves measuring the ability of substances to act as hydrogen providers or free-radical scavengers by interacting with the stabilized free radical DPPH[1]. Figure 1 shows the DPPH radical scavenging activity of aqueous (D/W) and hydro alcoholic (HA) plant extract compared to ascorbic acid (AA). As concentration increases, the antioxidant activity increases. The aqueous and hydro alcoholic leaf extracts show similar activity as compared to standard ascorbic acid.

Reducing power assay: The FRAP method relies on antioxidants capability to convert Fe^{3+} ions to $Fe^{2+,}$ aligning the antioxidant strength with this reducing ability. The introduction of TCA solution causes the precipitation of the potassium ferrocyanide complex and the addition of $FeCl_3$ results in the formation of a green to blue complex known as Berlin blue. Measurement of the color complex at 700 nm allows the determination of the reduction ability, indicating the potential antioxidant activity of compounds. Compounds with strong reduction abilities are likely to function as antioxidants by stabilizing radicals through electron or hydrogen atom donation, enhancing the stability of radical compounds[22].

Figure 2 shows the reductive capabilities of aqueous(D/W) and hydro alcoholic (HA) plant extracts compared to ascorbic acid (AA). Ferric ion reducing antioxidant power activity shows that as the concentration increases, the reducing power also seems to increase. However, the aqueous and hydro alcoholic leaf extracts show less activity as compared to standard ascorbic acid.

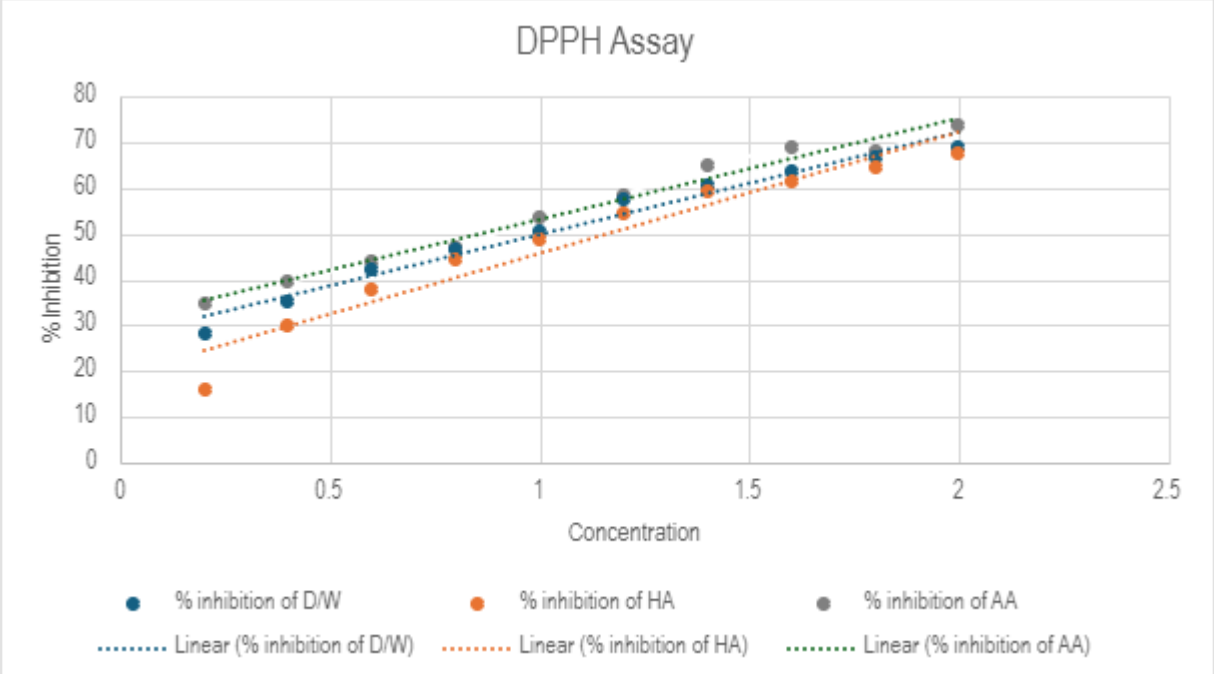

Figure 1: Free radical scavenging activity of *Mitragyna parvifolia* leaf extract

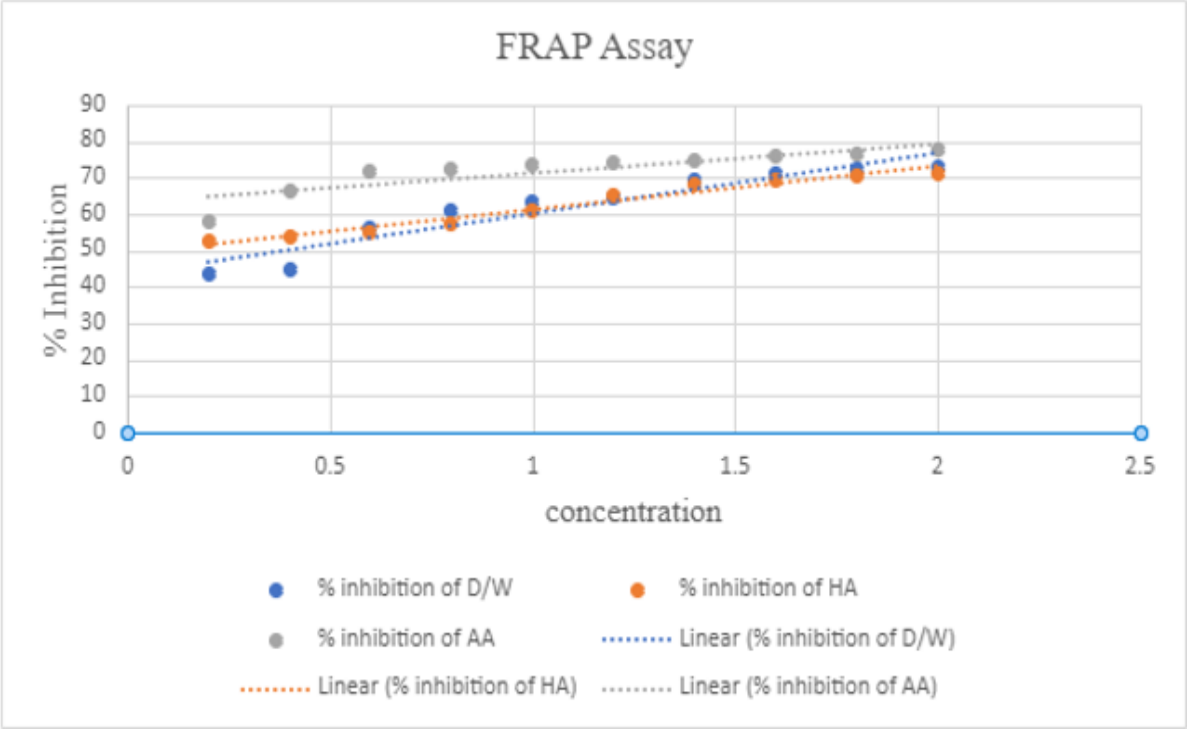

Figure 2: Reducing power assay of *Mitragyna parvifolia* leaf extract

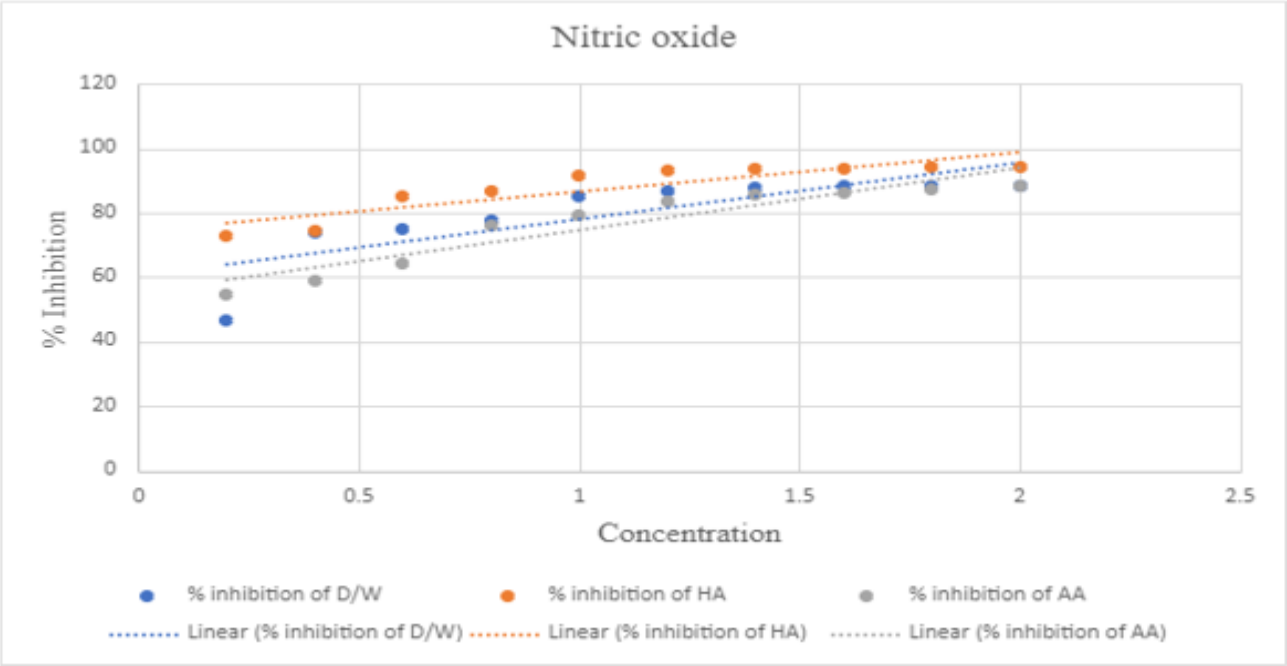

Figure 3: Nitric oxide scavenging activity of *Mitragyna parvifolia* leaf extract

Nitric oxide scavenging activity: Nitric oxide (NO) is a highly reactive diatomic free radical with a brief lifespan in biological systems. An indirect method to determine NO spectrophotometrically involves measuring its stable decomposition products NO_3 and NO_2. This process entails reducing NO_3 to NO_2 followed by determining NO_2, through the Griess reaction. The Griess reaction involves a two-step diazotization reaction where the nitrosating agent N_2O_3 reacts with sulfanilamide, forming a diazonium ion. This ion couples with N-(1-naphthyl) ethylenediamine, producing a chromophoric azo product with strong absorption at 540 nm. Enzymatic reduction of NO_3 to NO_2 using a commercially available nitrate reductase has proven to be an effective method for quantifying these compounds in extracellular fluids[2]. Figure 3 shows the nitric oxide scavenging activity of aqueous (D/W) and hydro alcoholic (HA) plant extract compared to ascorbic acid (AA). Nitric oxide scavenging activity shows that as the concentration increases, the activity goes on increasing rapidly, but as the concentration reaches 1µg/ml and above, the activity does not increase exponentially. However, the activity of aqueous and hydro alcoholic leaf extract is greater than standard ascorbic acid.

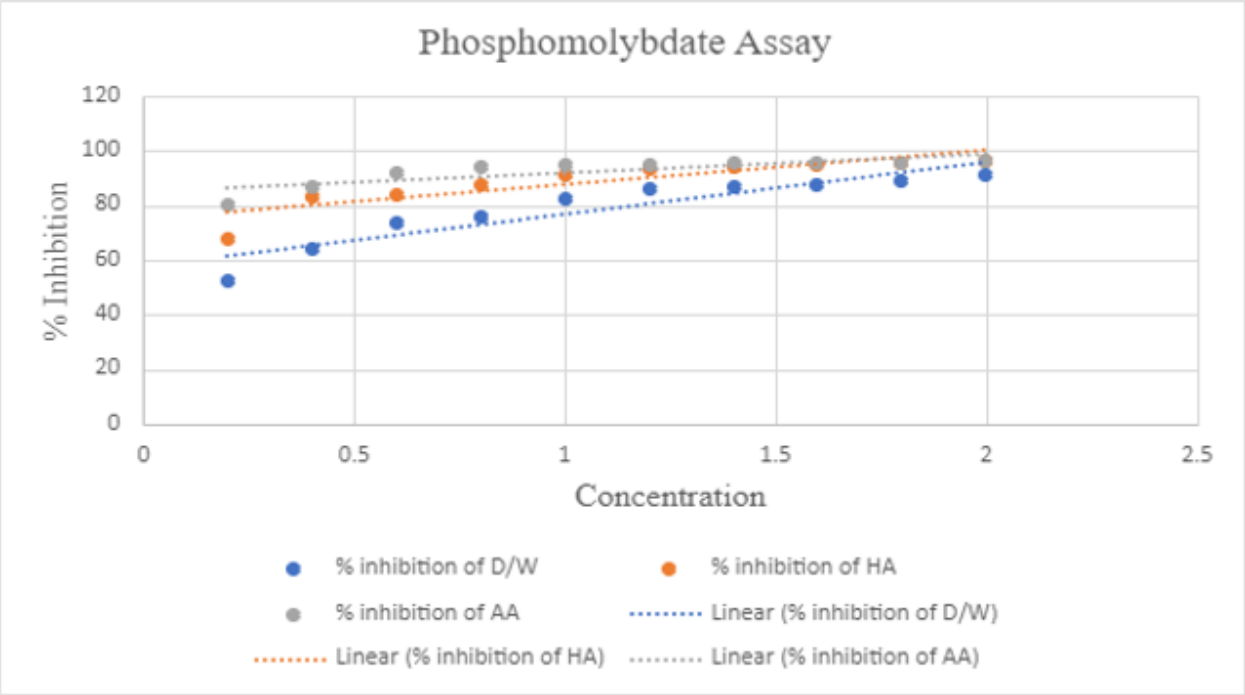

Figure 4: Phosphomolybdenum assay of *Mitragyna parvifolia* leaf extract

Phosphomolybdenum assay: This assay involves the extract reducing Mo (VI) to Mo (V), leading to the formation of a green phosphate/Mo (V) complex under acidic pH conditions[14]. Figure 4 shows the reducing Mo (VI) to Mo (V) activity of aqueous (D/W) and hydro alcoholic (HA) plant extract compared to ascorbic acid (AA). The percentage total antioxidant activity shows an increase in the activity as the concentration increases. In comparison to standard ascorbic acid and hydro alcoholic leaf extract, the aqueous leaf extract has lower activity. However, the hydro alcoholic leaf extract has comparable activity with standard ascorbic acid.

Conclusion

The study demonstrates that both aqueous (D/W) and hydro alcoholic (HA) plant extracts exhibit significant antioxidant activities as evidenced by their performance in DPPH, FRAP, nitric oxide scavenging and phosphomolybdenum reduction assays. The antioxidant activity of the plant extracts generally increases with concentration. The DPPH assay shows that both extracts have similar scavenging activity to ascorbic acid whereas the FRAP assay indicates a lower reducing power for the extracts compared to ascorbic acid.

The nitric oxide scavenging assay indicates that both extracts show greater scavenging activity than ascorbic acid at higher concentrations while the phosphomolybdenum assay shows that the hydro alcoholic extract has comparable activity to ascorbic acid and the aqueous extract exhibits lower activity. These results suggest that the hydro alcoholic extract, in particular, may serve as an effective antioxidant. The result of the antioxidant activity of leaves of *Mitragyna parvifolia* highlights its potential as a natural source of antioxidants. Further research is warranted to explore the practical applications of *Mitragyna parvifolia* in preventing oxidative stress-related diseases and developing antioxidant-rich pharmaceutical or nutraceutical products.

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