IN-VITRO ANTIOXIDANT ACTIVITY of HYDROALCOHOLIC EXTRACT of FRUITS of *ZANTHOXYLUM RHETSA* (ROXB.) DC

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I. Abstract

Free radicals can interact with lipids, nucleic acids, proteins and enzymes, leading to cellular damage. Such oxidative stress is implicated in aging and in the pathogenesis of several degenerative disorders, including cancer, cardiovascular disease, cataracts, liver dysfunction, diabetes mellitus, inflammation, and renal failure. Although the human body possesses an inherent antioxidant defence system, the endogenous levels are often insufficient to counteract excessive free radical production. Therefore, supplementation with dietary antioxidants is considered essential and plant-derived "natural antioxidants" have gained considerable attention in food science and preventive medicine. The present study investigates the antioxidant potential of hydroalcoholic fruit extracts of *Zanthoxylum rhetsa* (Roxb.) DC. Antioxidant activity was evaluated using three in vitro assays: 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Nitric Oxide Radical Scavenging. The extract demonstrated strong radical scavenging activity in a dose-dependent manner, with maximum potency observed in DPPH and Nitric oxide assays, followed by FRAP (DPPH > Nitric oxide > FRAP). These findings indicate that *Zanthoxylum rhetsa* fruits are a promising source of natural antioxidants

II. Introduction

Herbal medicines have been used since ancient times to alleviate disease symptoms. Despite significant advancements in modern medicine in recent decades, medicinal plants continue to play a vital role in healthcare. Their widespread use, particularly in traditional medicine, is largely attributed to their long-standing application in folk remedies and their preventive health benefits, especially in developing nations (Saeed *et al*., 2012).

Oxidative stress (OS) occurs when there is an imbalance between the generation of reactive oxygen species (ROS) and the cell's ability to neutralize them. It has been identified as a key factor in the development of

various diseases, including cancer, diabetes and cardiovascular disorders. ROS can inflict damage on essential cellular components such as lipids, proteins and nucleic acids like DNA, ultimately leading to cell death through necrosis or apoptosis. This damage can be further exacerbated by a compromised cellular antioxidant defence system. All living organisms possess intrinsic antioxidant mechanisms that help protect against oxidative damage and include repair enzymes that eliminate damaged molecules. However, these natural defence systems may sometimes be insufficient, making dietary intake of antioxidant-rich compounds crucial for maintaining cellular health (Khan *et al.*, 2013). Numerous medicinal plants have been extensively studied for their antioxidant properties. Natural antioxidants, whether in the form of crude extracts or isolated bioactive compounds, are highly effective in preventing damage caused by oxidative stress (Saeed *et al*., 2012).

The plant of emphasis in the research belongs to family Rutaceae, *Zanthoxylum rhetsa* (Roxb.) DC. *Zanthoxylum rhetsa* is a shrub or tree that sometimes grows to a height of 26 m (85 ft). The plant is sometimes deciduous and has stems with thick, cone-shaped spines on the older stems. The leaves are 140-230 mm long and pinnate, with nine to twenty-three egg-shaped to elliptical leaflets. The flowers are arranged on the ends of branch lets, sometimes also in leaf axils, in panicles up to 150 mm long. The herb is well distributed in tropical and subtropical regions in Africa and other part of the world. The fruits and seeds of *Zanthoxylum rhetsa* are used to treat toothache, dizziness and bloating, malaria, urinary diseases and rheumatism. The species has also been reported to be routinely used as food in some Asian countries, for example; the shoots are consumed as food by indigenous people of northeast India, and the Northern Thai people use the fruit as a spice, and an appetizer especially in pork salad and curry. The *Zanthoxylum* genus is well known to contain phytochemicals which are responsible for its medicinal/therapeutic properties, particularly, Characteristic secondary metabolites of *Zanthoxylum* species include lignoids, alkaloids, amides, flavonoids, terpenes, sterols and coumarins. The plant has been reported to exhibit antimalarial, antimicrobial, anticancer, antidiarrheal, antioxidant, antiviral activities and many more therapeutic activities (Maduka *et al.,* 2021).

In this study the Hydro-alcholic extract of *Zanthoxylum rhetsa* (Roxb.) DC. fruits are tested for its anti-inflammatory properties using in-vitro methods like Free radical scavenging activity (DPPH), Reducing power (FRAP) and Nitric oxide scavenging activity assays respectively. This research seeks to explore the anti-inflammatory potential of the *Zanthoxylum rhetsa* fruits which can be used as an anti-inflammatory source having very less to no side effects as compared to the synthetic products found in the market which causes long term side effects.

III. Materials and Methods

The fruits of the plant material were collected from the local market situated in Mumbai, India. They were authenticated from the Botanical Survey of India, 7-Koregaon road Pune- 411001. The fruits were air dried and the seeds were removed and separated. The pericarp was air dried again till all the moisture had been removed and then were ground to a fine powder. Hydro-alcohol (30:70) was used as the solvent for extraction by cold maceration method. The antioxidant activity of the plant extract and standard were assessed based on radical scavenging effect of the stable DPPH free radical according to method of (Blois,1958). The reducing power of the hydroalcoholic extract was determined according to the method of (Oyaizu,1986). Nitric oxide was generated from nitroprusside and measured by the Griess reaction (Marcocci *et al.,* 1994).

IV. Result & Discussion

A. Free radical scavenging activity (DPPH)

DPPH. is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Sakanaka *et al.*, 2005). The assay is based on the reduction of DPPH radical in hydro-alcohol (30:70) which causes an absorbance drop at 517 nm. DPPH is known to abstract labile hydrogen (Matsubara *et al*., 1991). DPPH being a stable free radical can accept an electron or hydrogen radical to become stable diamagnetic molecule. Due to its odd electron, the solution of hydro-alcoholic DPPH shows

a strong absorption band at 517 nm; reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses color stoichometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compounds/ plant extracts to act as a free radical scavenger. Antioxidant molecules can quench DPPH free radicals and convert them to a colorless/bleached product. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of extract in terms of hydrogen atom-donating capacity (Joshi *et al*., 2023). Figure 1. shows the dose responses curve of DPPH radical scavenging activity of hydroalcoholic extract, compared with ascorbic acid, as standard.

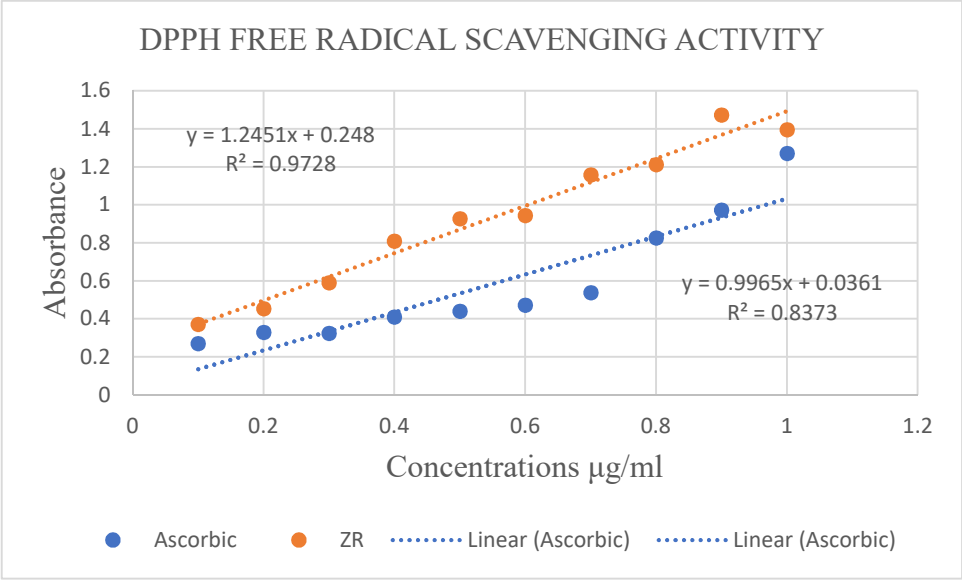

Fig 1. DPPH free radical scavenging activity of Hydroalcoholic extract

B. Reducing power assay (FRAP)

The reducing power of the extracts was measured by the direct electron donation in the 3+ reduction of $[Fe^{3+}(CN)_6]^{3-}$ to $[Fe^{2+}(CN)_6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the reduction reaction, by forming the intense Prussian blue colour complex, $(Fe^{3+})_4[Fe^{2+}(CN)_6]_3$, and quantified by absorbance measurement at 700 nm. The reducing capacity of *Zanthoxylum rhetsa* might serve as a significant indicator of its potential antioxidant activity. A direct correlation between antioxidant activity

and reducing power of certain plant extracts has been observed. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1998). Figure 2 shows the reductive capabilities of the plant extract compared to ascorbic acid. The reducing power of hydro-alcholic extract of Zanthoxylum rhetsa (HAZR) was very potent and the power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of ascorbic acid. Figure 2. shows the reducing power assay of hydroalcoholic extract, compared with ascorbic acid, as standard.

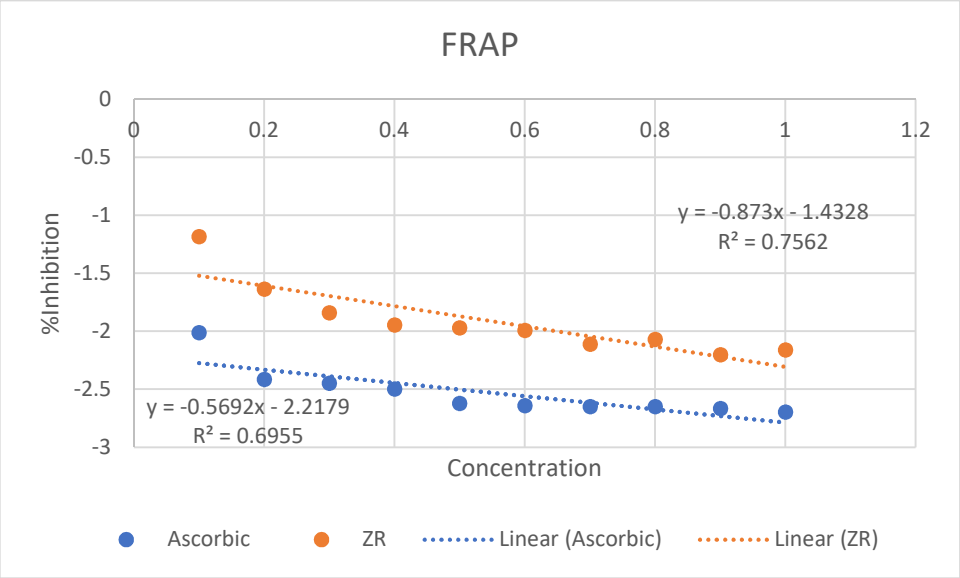

Fig 2. FRAP reducing power assay of hydroalcoholic extract

C. Nitric oxide scavenging activity

Nitric oxide is an important radical in biological systems, which serves many important biological functions as an intra and intercellular messenger. As a free radical, it is oxidized, reduced or complexed with other biomolecules, depending on microenvironment (Moncada *et al.,* 1991). These reactive nitrogen species are implicated in inflammation, cancer and other pathological conditions. The reactivity of NO is enhanced by oxygen (O_2) through the conversion in reactive intermediates including nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$). Hence, the extract was evaluated for their ability to scavenge

the NO radical. Nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite (Sreejayan *et al*., 1991). Figure 3. shows the moderately good nitric oxide scavenging activity of hydroalcoholic extract, compared with ascorbic acid, as standard.

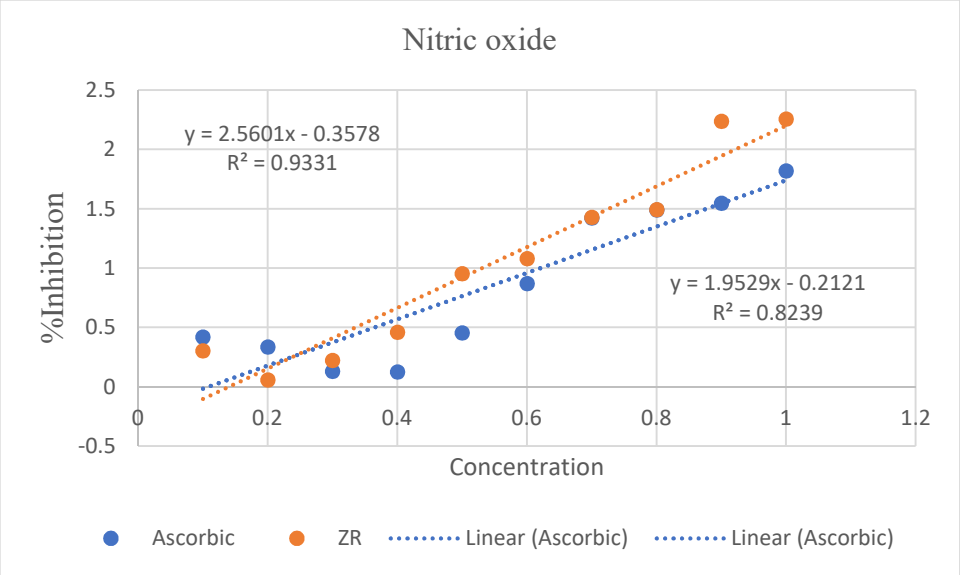

Fig 3. Nitric oxide scavenging activity of hydroalcoholic extract

V. SUMMARY

The anti-oxidant activity of the hydro-alcoholic extract was examined in comparison to the reference; in all the three methods. Higher antioxidant activity is shown by the DPPH and FRAP assays, which are followed by nitric oxide assay. DPPH> Nitric oxide> FRAP.

VI. CONCLUSION

Based on the results in the above data, it can be concluded that the hydro-alcoholic extract of *Zanthoxylum rhetsa* fruits exhibits high antioxidant and free radical scavenging activities. It also chelates iron and has reducing power. These in-vitro assays indicate that this plant fruit extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stress.

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