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An Invitro Studies on Antioxidant and Wound Healing Activity of Fruit Peel Extracts of *Moringa oleifera* Lam.

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Abstract:

Moringa oleifera commonly referred to as the "miracle tree," is renowned for its robust nutritional profile and medicinal properties, thriving across tropical and subtropical regions globally. Although native to Afghanistan, Bangladesh, India, and Pakistan, this versatile tree is celebrated for its cost-effective contribution to nutrition. Extensive research has highlighted the utility of various parts of the *M. oleifera* tree: Bark: Utilized in the treatment of ulcers, toothache, and hypertension. Roots: Effective against toothache, helminthiasis (parasitic worm infections), and paralysis. Flowers: Employed in the treatment of ulcers, enlarged spleen, and as aphrodisiacs. However, the medicinal potential of the fruit peel had not been thoroughly investigated until recent studies explored its properties. These studies aimed to analyze the antioxidant and wound healing properties of *M. oleifera* fruit peel extracts, prepared using water and ethanol as solvents. The findings from these investigations are promising. The extracts from *M. oleifera* fruit peel demonstrated significant antioxidant activity, suggesting a rich presence of compounds capable of neutralizing harmful free radicals. Furthermore, the extracts exhibited notable wound healing properties, potentially offering a natural and cost-effective option for promoting wound repair and recovery. In conclusion, the fruit peel of *Moringa oleifera*, an often-overlooked part of the tree, possesses valuable antioxidant and wound healing properties.

These findings enhance the tree's already impressive medicinal profile and open new avenues for its use in natural health remedies.

Keywords: *Moringa oleifera* Lam, Antioxidant, Wound Healing, Free Radicals, Fruit Peel.

1. Introduction

Moringa oleifera Lam. (*M. oleifera)* is extensively distributed and utilized in tropical and sub-tropical regions worldwide, primarily indigenous to India and Africa. Commonly known as the drumstick tree or horseradish tree, it's also hailed as the "miracle tree," "natural gift," or "mother's best friend" due to its highly nutritious leaves containing protein, minerals, and βcarotene (Leone

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et al., 2015). *M. oleifera* leaves are versatile in consumption and retain their nutrient potency for extended periods when dried into powder form. Consequently, regional and international relief organizations are increasingly focusing on harnessing *M. oleifera* leaves as a nutritional supplement in various African countries (Rodrigues-Perez et al., 2015).

Traditionally, in nations like India, Pakistan, and Uganda, *M. oleifera* has been utilized extensively to alleviate diverse ailments including diabetes, obesity, hysteria, scurvy, and even tumors (Gupta et al., 2018; Kasolo et al., 2010). It's reported that *M. oleifera* contains numerous phytoconstituents such as flavonoids (Coppin et al., 2013), alkaloids (Panda et al., 2013), saponins (Mathur et al., 2014), saccharides (Roy et al., 2007), glucosinolates (Maldini et al., 2014), tannins (Bhatta et al., 2012), phenolic acids (Coz-Bolanos et al., 2018), and nitrile glucosides (Sahakitpichan et al., 2011). These complex natural compounds contribute to its myriad pharmacological activities.

For instance, *M. oleifera* leaves demonstrate notable anti-inflammatory (Coppin et al., 2013), anti-cancer (Jung et al., 2014), antioxidant (Verma et al., 2009), antibacterial (Peixoto et al., 2011), hepatoprotective (Atta et al., 2018), cardioprotective (Panda et al., 2013), antihypertensive (Dangi et al., 2008), hypolipidemic (Helmy et al., 2017), and hypoglycemic (Jaiswal et al., 2009) properties. Similarly, the seeds exhibit distinct antimicrobial (Singh et al., 2013), antidiabetic (Al-Malki et al., 2015), and anti-inflammatory (Araujo et al., 2013) activities, while the roots may possess anti-inflammatory (Cui et al., 2019), anti-cancer (Ghosh et al., 2016), antiulcer (Choudhary et al., 2013), antifertility (Shukla et al., 1988), and antiurolithiatic (Karadi et al., 2006) effects.

Consequently, *M. oleifera* has garnered significant attention in recent years due to its immense potential as a source of both nutritious food and medicinal value. Although there's a plethora of research focusing on the antioxidant and anti-inflammatory properties of *M. oleifera* leaves, few studies have explored other parts of the plant. For instance, Ndhlala et al. (2014) investigated the antioxidant activities of leaf extracts from thirteen *M. oleifera* cultivars from various regions, while Siddhuraju et al. (2003) examined antioxidant activities across different agroclimatic regions. Coppin et al. (2013) compared the anti-inflammatory activities of *M. oleifera* leaves from sub-Saharan Africa. However, there's a gap in research regarding the characterization of *M. oleifera* collected in Kenya, particularly in a systematic comparison of phytochemicals across its different organs (leaves, seeds, and roots) and their correlation with various biological activities.

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Furthermore, while numerous reports focus on the antioxidant and anti-inflammatory activity of *M. oleifera* leaves, there's a dearth of information regarding the antioxidant and wound healing property of the fruit peel. Hence, this study aims to investigate the antioxidant and wound healing property of *M. oleifera* fruit peel extracts.

2. Materials and Methods

2.1. Plant Materials

Moringa oleifera (Drumstick) fruit was purchased from the local vegetable market near Ambattur, Chennai. Fruits were cleaned, skin was peeled. Then fruit peels were sun dried. The dried fruit peels were prepared by powdering each individually and then sieved. Then the extraction process, infusion, a type of maceration was utilized for the extract preparation. After sieving, the fine powder was placed in a clean container. Then the ethanol (50%) is poured on top of the fine powder, soaked, and kept for a short period of time (2h) to extract the bioactive constituents that are readily soluble. Simultaneously, aqueous extract was prepared using water. Then, the samples were filtered with grade Whatman No. 1 filter paper. The extract prepared was 2% and; they were stored and utilized for further analysis.

2.1. Ferric reducing antioxidant power assay:

The antioxidant capacity of the extract was estimated spectrophotometrically following the procedure of Benzie and Strain (Benzie and Strain, 1996). The method is based on the reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+} -tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm.

The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°. Freshly prepared working FRAP reagent was pipetted using 1-5 ml variable micropipette (3.995 ml) and mixed with 5 µl of the appropriately diluted plant sample and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 ml FRAP reagent+5 µl distilled water) after 30 min incubation at 37°. All the determinations were performed in triplicates.

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2.2. Determination of Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of extract was determined by Griess Ilosvay reaction using sodium nitroprusside (Can *et al*., 2022). In a typical experiment, the reaction mixture containing 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer (pH-7.4) was mixed with 0.5 mL of samples and vitamin-C (standard); then incubated for 150 min at 25 °C. After the incubation period was over, 0.5 mL of nitrite was pipetted out and 1mL of sulfanilic acid reagent (0.33% of sulfanilic acid in 2% glacial acetic acid) was added to it and kept for 5 min. Then, 1 mL of 1% naphthyl ethylene diamine dihydrochloride (NEDD) was added and allowed to stand for 30 min at 25 °C. The absorbance of pink colour of the solution was read at 540 nm. The percentage of nitric oxide inhibition was calculated using the following equation:

Percentage (%) of nitric oxide radical scavenging assay = $[(A_0-A_1)/A_0] \times 100$.

Where A_0 - absorbance of control, & A_1 - absorbance of the treated sample.

Statistical Analysis

In this study, all the experiments were carried out in three independent biological replicates, and data were reported as mean ± standard deviation for each set of conditions. Statistical significance of the data was tested through one - way analysis of variance (ANOVA) using least significant difference with $p<0.05$, $p<0.01$, $p<0.001$.

3. Results

Fig 1: Antioxidant activity of aqueous & ethanolic extract of drumstick fruit peel. (n=5)

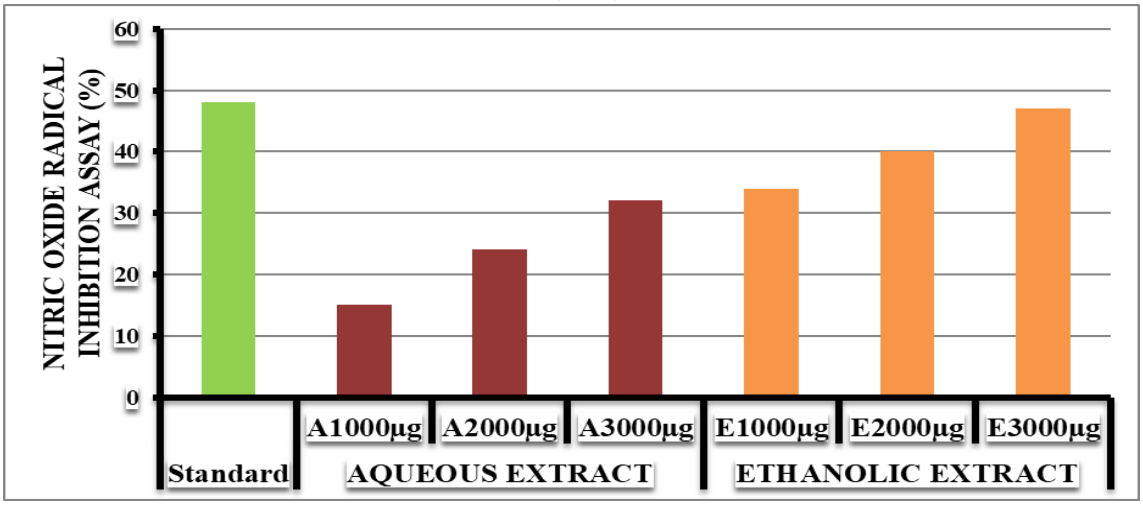

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Figure. 1a: Nitric oxide radical inhibition assay (%)

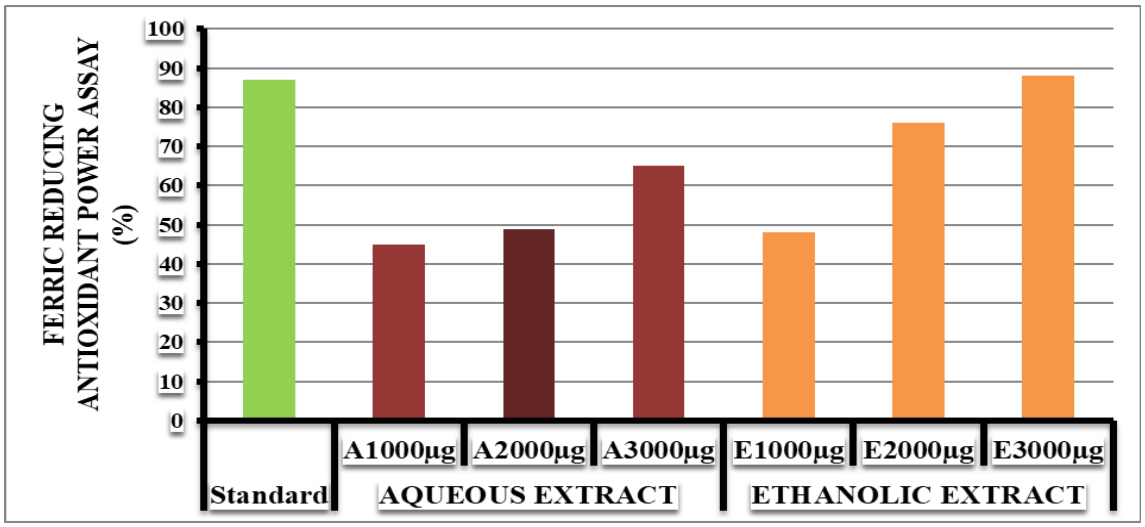

Figure. 1b: Ferric reducing antioxidant power assay (%)

Figure 1a and 1b depicts the antioxidant activity of drumstick fruit peel which showed the gradual increase in antioxidant activity towards ferric ion and nitric oxide radical for both aqueous and ethanolic extracts. In addition, it showed the comparatively increased antioxidant activity for ethanolic extracts of drumstick fruit peel compared to aqueous extract.

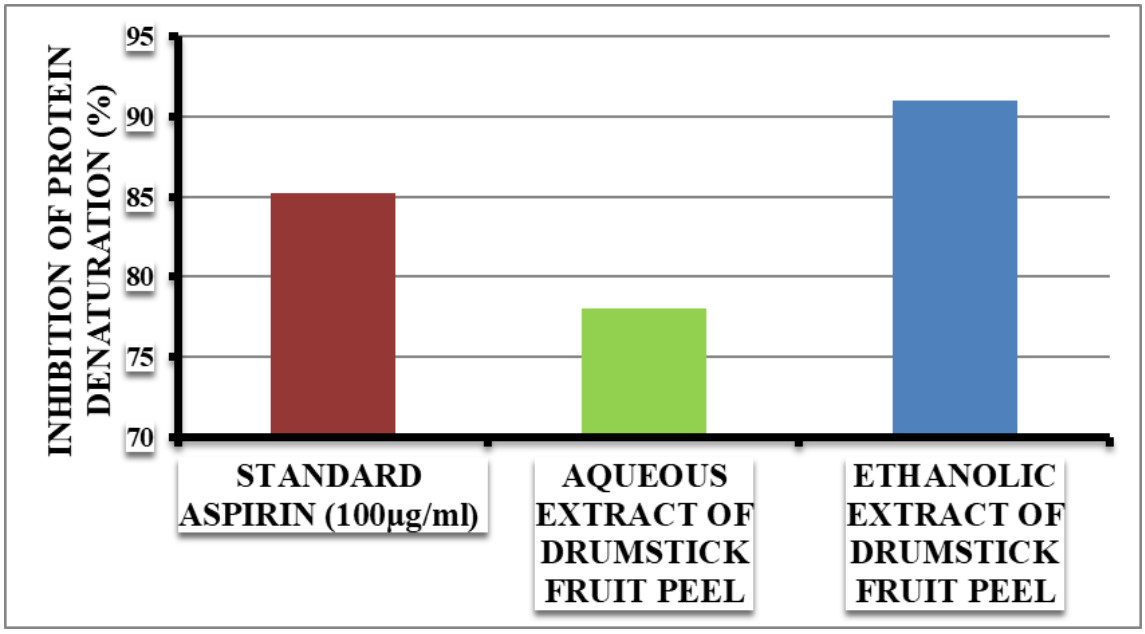

Figure 2: Wound healing property of aqueous and ethanolic extract of drumstick fruit peel by protein denaturation inhibition assay. (n=5)

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Figure 2 depicts the wound healing property of aqueous and ethanolic extract of drumstick fruit peel which showed the significant increase in inhibition of protein denaturation by ethanolic extract compared to standard aspirin and aqueous extract of drumstick fruit peel.

4. Discussion

Moringa oleifera is a medicinal plant that ethnobotanical studies have shown its inclusion in treatment of many ailments such as diarrhea, diabetes, epilepsy, wound healing and arthritis. It is a plant that was believed to originate from India but could now be found in both the tropics and the sub-topics.

Antioxidants are well known for their role in deactivating free radicals that have damaging roles to biological cells. The most prevalent antioxidants seen in plants are polyphenolic compounds, which are the secondary plant metabolites that arise from a common intermediate, phenylalanine, or its close precursor, shikimic acid. Polyphenolic compounds are categorized into four main types namely flavonoids, phenolic acids, stilbenes and lignans. Evidences from epidemiologic studies have revealed that consumption of leafy vegetables was associated with reduced risk of diseases due to the presence of their antioxidant properties. Previous studies have demonstrated that the antioxidant activity of plant materials is positively correlated to their phenolic components which thus indicate its antioxidant activity.

Many pharmacological studies have shown the ability of this plant to exhibit analgesic, anti-inflammatory, antipyretic, anticancer, antioxidant, nootropic, hepatoprotective, gastroprotective, anti-ulcer, cardiovascular, anti-obesity, antiepileptic, antiasthmatic, antidiabetic, anti-urolithiatic, diuretic, local anesthetic, anti-allergic, anthelmintic, wound healing, antimicrobial, immunomodulatory, and antidiarrheal properties. In this, *Moringa* fruit peel extracts were prepared and tested for their antioxidant properties. Figure 1A and 1B depicts the antioxidant activity of drumstick fruit peel which showed the gradual increase in antioxidant activity towards ferric ion and nitric oxide radical for both aqueous and ethanolic extracts. In addition, it showed the comparatively increased antioxidant activity for ethanolic extracts of drumstick fruit peel compared to aqueous extract. Higher antioxidant activity of ethanolic extract explains the comparatively increased solubility nature of phytochemicals in organic solvent, ethanol. The different compounds can be extracted with different solvents due to their different solubility characteristics.

However, insignificant difference was observed between aqueous and ethanolic extract showed that both can be utilized for further validation. In agreement to our study, MO fruits and leaves were demonstrated to have antioxidant properties (Luqman *et al*., 2012). Extract of leaf showed a concentration-dependent increase in glutathione level and a decrease in malondialdehyde level, fruit extract showed beneficial results in eliminating free radicals, extract of roots significantly reduced iron and FeSO4-induced microsomal lipid peroxidation in a dose-dependent manner (Sinha *et al*., 2011; Satish *et al*., 2014).Similarly, pods were also found to be capable of scavenging peroxyl, superoxyl, and 2, 2-diphenyl-2-picryl hydrazyl (DPPH) radicals (Paliwal *et al*., 2011).

Besides displaying antioxidant activity, MO leaf extract also showed a dosedependent nephroprotective action in an acetaminophen-induced nephrotoxicity model in male BALB/c rats (Karthivashan*et al*., 2016). Triterpenoids, moringyne, monopalmitic and di-oleic triglyceride, campesterol, stigmasterol, β-sitosterol, avenasterol, vitamin A, and its precursor beta-carotene have been shown to contribute for antioxidant properties (Stavros and John, 2002) in pods, leaves and fruits of *Moringa*.

In the context of wound healing property, Figure 2 depicts the wound healing property of aqueous and ethanolic extract of drumstick fruit peel which showed the significant increase in inhibition of protein denaturation by ethanolic extract compared to standard aspirin and aqueous extract of drumstick fruit peel.

Consistent to our study results, various parts of *Moringa* plant were tested for their wound healing property in vitro and in vivo. Extracts of leaf, dried pulp, and seeds showed a significant increase in hydroxyproline content, wound-closure rate, granuloma-breaking strength, and granuloma dry weight, and a decrease in scar area and skin-breaking strength in incision, excision, and dead space wound models in rats (Lambole and Kumar, 2012). Studies conducted on the effect of wound healing of leaf extract in diabetic animals showed improved tissue regeneration, decreased wound size, downregulated inflammatory mediators, and upregulated vascular endothelial growth factor in wound tissues,and remarkable antiproliferative and anti-migratory effects on normal human dermal fibroblasts (Muhammad*et al*., 2016; Gothai*et al*., 2016). Results in the context of wound healing property suggest that accumulation of collagen content and underlying wound healing mechanism through

antimicrobial, antioxidant, and anti-inflammatory activities may be contributed by its bioactive phytochemical content, which has the potential to accelerate the wound contraction, increase the rate of epithelialization, and protect tissues against oxidative damage. Antioxidant activity is important because it can intervene in the inflammation tissue damage, which is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites which well supports our study results where increased antioxidant activity and the accompanied wound healing property was observed. According to Hosseinkhani*et al*. (2017), antioxidant properties were found in Persian medicine used for wound healing, which are *Cocos nucifera L., Commiphoramukul (Hook ex Stocks) Engl, Gentiana lutea L., Teucrium polium L., Punica granatum L. Plantago major L., Adiantum capillus-veneris L., Aloe vera (L.) Burm f, and Potentilla reptans L*.

Overall, our results suggest that *Moringa oleifera* fruit peel extract exhibit a significant antioxidant as well as wound healing property. However, further research studies are required to acquire deep understanding of phytochemicals present in Moringa fruit peel extracts which may aid in the recommendation of Moringa fruit peel as dietary supplement or for its utilization in pharmacological preparations. Then, it can be utilized for the management and treatment of various ailments. In addition, the results confirm the use of the plant in traditional medicine. Now our study will be directed to explore the lead compound responsible for aforementioned activity from this plant. Based on these fruitful findings, further investigation on the aqueous / ethanolic bioactive characterization and fractionization is highly recommended to identify bioactive compounds.

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