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ANTI-OXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT, MINERAL, VITAMIN AND PHYTOCHEMICALS ANALYSIS OF NEEM FRUIT PULP AND NEEM SEEDS

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ABSTRACT
Azadirachta indica, which was the scientific name of Neem that belongs to Meliaceae family, mostly found in South Asia (India, Pakistan, Bangladesh and Nepal). The average height of Neem includes 29m to 30m with the diameter of 4-5feets. Leaves are imparipinnately arranged with 5 to 15 leaflets and the fruits are yellow-green drupes. The study of Neem is to determine the antioxidant activity, nutritional analysis (vitamin C, vitamin E, Mg, Ca and Fe), phytochemical analysis (total phenolic content, alkaloids, flavonoids, saponins, tannins and phytates), anti-diabetic and anti-microbial activity. The results of Neem fruit showed as Antioxidant activity by FRAP is 9.797micromol, by DPPH is 1279ppm and by ABTS is 76.05 GAE/g of inhibition against free radicals. The vitamin C is 1.73mg, vitamin E is 49mg, magnesium is 45mg, calcium is 7.3mg, iron is 102mg and potassium is 89mg. Antimicrobial activity is 100CFU/g and total phenolic content is 66.32GAE/g. Antidiabetic activity is high due to carbohydrates present in it. Saponins are more in number than tannins, alkaloids, flavonoids and phytates. Neem helps to maintain liver, kidney, hair, dental, skin and heart health. Neem is used as an insect and mite repellents and also as pesticides. Neem has anti-inflammatory, anti-arthritic, anti-pyretic, anti-fungal, anti-bacterial, anti-tumour and anti-fertile properties.

KEYWORDS: Neem Seed, Neem Pulp, Antioxidant Activity, Nutritional Analysis, Total Phenolic Content.

INTRODUCTION
Neem (Azadirachta indica), a member of the Meliaceae family, is a significant medicinal plant native to South Asia, including India, Pakistan, Bangladesh, and Nepal. Known for its extensive therapeutic properties, Neem has been utilized in various traditional medicine systems such as Unani, Ayurveda, and Homeopathy, and is reputed to be one of the oldest medicinal systems due to its adaptability to diverse agro-climatic conditions (Subendu Sarkar et al., 2021; Mohammad A. Alzohairy, 2016).

This versatile tree is widely cultivated across South Asia and parts of Africa. Neem's medicinal benefits are attributed to various parts of the tree—leaves, bark, fruit, flowers, oil, and gums. These components are employed in the management of several medical conditions, including cancer, hypertension, heart disease, and diabetes (Jose Francisco Islas, 2020). Neem trees can grow up to 30 meters tall and are characterized by their broad, white, bisexual flowers and evergreen leaves. They are resilient to extreme drought and can thrive in poor, rocky soils, though they do not withstand freezing temperatures or waterlogging (Melissa Petruzzello, 2019).

In practical applications, Neem seed extract is widely used in shampoos for lice treatment, oral care products for plaque control, and prevention of gingivitis (Alena Clark, 2024). The strong-smelling, sulfur-rich yellow neem seed oil, although effective against lice, is not recommended for ingestion due to its bitterness (Nicole Galan, 2019). Furthermore, recent advancements have utilized neem extracts to develop silver and titanium dioxide nanoparticles aimed at targeting cancer cells selectively, preserving healthy cells (Shivani Rana and Akhil Saxena, 2023).

Neem's role extends beyond medicinal uses; it is also an effective afforestation tool that mitigates soil erosion, reduces temperatures, and addresses various environmental concerns by providing shade, firewood,

and improving soil conditions (Nandita Mandi et al., 2022). The tree's bioactive compounds include isoprenoids, triterpenoids, polysaccharides, and polyphenols, with bitterness linked to the accumulation of limonoids such as nimbin, nimbidin, and nimbolide (Subendu Sarkar et al., 2021; Mohammad A. Alzohairy, 2016). These compounds are vital for their roles in disease management, including anti-inflammatory, anti-arthritic, anti-pyretic, anti-fungal, anti-bacterial, and anti-tumor activities.

Neem extracts contribute to molecular mechanisms such as DNA repair, scavenging, and immune surveillance, enhancing anti-inflammatory and anti-angiogenic activities and modulating signaling pathways (Jose Francisco Islas, 2020). The high polyphenol content in neem extracts provides antioxidant activity, which is beneficial in preventing liver damage induced by medications like aceclofenac (Subendu Sarkar et al., 2021).

In addition to its medicinal uses, neem is incorporated into personal care products like shampoos, soaps, and toothpaste for treating skin conditions such as acne, psoriasis, and athlete's foot. Its low toxicity profile makes it suitable for organic farming (Melissa Petruzzello, 2019). Neem is also used for treating wounds, promoting kidney health, improving hair and dental health, and fighting plaque buildup on teeth (Fatima Hallal, 2021; Alena Clark, 2024). However, neem can cause allergic reactions and other adverse effects, and its consumption is cautioned against in pregnant and lactating women due to its bitter taste and potential toxicity (Alena Clark, 2024). Moreover, neem presents an alternative to vasectomy by preventing sperm release in men, highlighting its diverse applications in reproductive health (Fatima Hallal, 2021).

MATERIALS AND METHODS

Based on the described background, there is potential for Neem pulp and seed to produce antioxidant activity and phytochemical study of Neem pulp and seed

Collection of Neem fruits and Seeds

In June 2024, neem fruits were collected from Pati ghanpur, Patancheru, Telangana. The small, oval fruits were washed, pulp collected, and seeds dried and powdered.

Fig 1: Neem Fruit.

Fig 1: Collection of Neem Fruit.

Solvent extraction of Neem fruit pulp

The neem pulp was weighed and added to 100ml of organic solvent, filtered, and centrifuged. The supernatant was then gathered, resulting in a volume of 40mcg, which was stored for further studies.

Solvent extraction of Neem seeds

The neem seed powder was weighed and added to 100ml of organic solvent, filtered, and centrifuged. The supernatant was then gathered, resulting in a volume of 40mcg, which was stored for further studies (G. L Preethi, 2023)

Determination of Vitamin C.

Sample preparation and evaluation of ascorbic acid by method spectrophotometer: The study utilized an Apple UV-visible spectrophotometer to analyze the ascorbic acid content in fruits and vegetables, homogenized with acetic acid, bromine water, thiourea, and oxidized ascorbic acid. The absorbance of each sample was measured to determine the content in fruit pulp and seeds. (Mohammad Idan Hassan AL Majidi, Hazim Y AL Qubury, 2016)

Vitamin E Analysis

The Association of Analytical Chemist (AOAC 1990) method was used to determine Vitamin E. Samples were weighted into a conical flask, added with ethanol, potassium hydroxide, and ascorbic acid. The flask was heated for 30 minutes at 40°C, then cooled and transferred to a separate funnel. The process involved adding petroleum ether to a beaker, shaking it, and adding it multiple times to extract phenolphthalein. The extract was then washed with water, filtered through anhydrous sodium sulphate, evaporated, and diluted with methanol. The supernatant was analyzed using High Performance Liquid Chromatography, using an Ultrasphere octadecylily (ODS) Hypersil column, methanol, and deionized water at pH 2.2. (Anna Rizzoli and Stefano Polesello, 1992)

PHYTOCHEMICALS STUDY

Total Phenolic content

The study involved calculating total phenolics using Folin-Ciocalteu reagent, centrifuging samples, homogenizing, precipitating, dilution, adding, and measuring absorbance in a UVD spectrometer for 60

minutes, presenting findings as mg catechol/100 g of fresh weight material.(G. Laxmi Preethi. 2023).

Test for Alkaloids

Ammoniacal chloroform was prepared by adding chloroform, ammonia, and sodium sulphate. Meyer's reagent was prepared by adding potassium mercuric iodide, mercury chloride, acetic acid, and water. A sample was weighed, added to a test tube, and sulphuric acid was added. The solution was tested with Mayer's reagent, and white precipitates were observed, indicating the presence of alkaloids. (Norazlonshah bin Hazali, et al, 2015).

Test for Flavonoid

A sample of five grams was weighed, chloroform added, and left in a fume hood overnight. Filtered and dried using a rotary evaporator, then added to a test tube with ether and 10% ammonia solution. The solution separated into two layers, with yellow in the ammonium layer indicating flavonoid presence and purple in the kuinon/juglon layer. (Norazlonshah bin Hazali, et al, 2015).

Test for Tannin

A 1% ferric chloride solution was prepared by adding 1.0g ferric chloride to 100ml distilled water. A sample was weighed, methanol added, and left in a fume hood overnight. Filtering was done, and a test tube was poured. The color change was observed, with blue-black indicating hydrolysable tannins and brownish-green indicating condensed tannins. (Norazlonshah bin Hazali, et al, 2015).

Test for Saponin

A sample of five grams was weighed, methanol added, and left in a fume hood overnight. Filtered through a funnel and paper, 1 ml of filtrate was poured into a test tube, and 5 ml of distilled water was added. The test tube was shaken for 30 seconds, and a stable froth was measured to determine the presence of saponin. (Norazlonshah bin Hazali, et al, 2015).

Antioxidant activity

ABTS cation radical-scavenging assay

The ABTS cation radical-scavenging assay was conducted using an UV/visible spectrophotometer and a temperature controller. A stock solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate, diluted with ethanol, and added to 3 ml of ABTS reagent. The absorbance readings were taken for 90 minutes until a plateau was reached. Total antioxidant capacity was calculated based on ascorbic acid reactivity, expressed as micrommol/g. (YY Soong, PJ. Barlow, 2004).

FRAP assay

The FRAP reagent was prepared using acetate buffer, TPTZ, and FeCl3, with a 10:1:1 ratio. The assay was conducted using 96 well plates and an automated microplate reader. The absorbance was recorded at 593nm for 4 minutes. Triplicate extracts were analyzed using the Trolox calibration curve, with values ranging from 2.5 to 33M per gram of dry weight.(Monika skowrts, 2014).

DPPH assay

Methanol extract was prepared in various concentrations (10,50, 100, 200, 400, 600g/ml) and ascorbic corrosive was used as a negative control. A DPPH solution was mixed with extract and standard solution, and the concentration was tested using a UV-Noticeable spectrophotometer. The lower absorbance of the reaction mixture indicated greater free radical-scavenging, calculated by multiplying the control's absorbance by the test sample's absorbance by 100. (G L. Preethi, 2023).

Estimation of Magnesium

About 20 ml of the food sample solution was neutralized with aqueous ammonia. 2 ml of buffer solution (NH4Cl-NH4OH), a drop of Eriochrome black -T indicator were added and titrated against EDTA solution until the colour change from wine red to blue. From the titre value the strength as well as the amount of Magnesium present in the whole of the sample solution was calculated.

Estimation of Calcium

Initially, calcium oxalate is precipitated out using ammonium oxalate. Dissolve this precipitate in concentrate sulphuric acid and calcium is calculated by Permanganometric titration.

Estimation of Iron

Dichromatometric titration was used to estimate Iron in food samples. From the titrate value the strength as well as the amount of Iron present in the whole of the food sample was calculated. (Mrinal Kanti si, et al, 2021.)

Statistical analysis

Statistical analysis used is two sample t test to compare between both the samples with the formula, t =(X1 – X2) / (S1^2/n1+S2^2/n2). (GL Preethi, 2023).

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity and vitamin content of Neem fruit pulp and seeds were evaluated using various assays and are summarized in Table 1.

Table 1: Antioxidant activity and Antioxidant rich vitamin content in Neem fruit pulp and seeds.

Methods and Antioxidant rich Vitamins	Neem pulp	Neem seeds
FRAP(micrommol Fe+/g)	45.50	12.30
DPPH (ppm)	72.75	40.10
ABTS(GAE/g)	2.5	1.5
Vitamin C (mg/100g)	1.73	0.9
Vitamin E (mg/100g)	1.5	0.6

The FRAP assay revealed that Neem fruit pulp exhibited a significantly higher antioxidant capacity, with 45.50 µmol Fe^{2+}/g compared to 12.30 µmol Fe^{2+}/g in Neem seeds. This indicates that Neem fruit pulp has a greater ability to reduce ferric ions, reflecting its potent antioxidant properties. Similarly, the DPPH assay results showed that Neem fruit pulp had a lower IC50 value of 72.75 ppm, demonstrating superior radical scavenging activity relative to Neem seeds, which had an IC50 value of 40.10 ppm. This suggests that the fruit pulp is more effective at neutralizing free radicals, thereby offering better protection against oxidative stress.

The ABTS assay further supports these findings, with Neem fruit pulp having a higher antioxidant activity of 2.5 mg GAE/g compared to 1.5 mg GAE/g in Neem seeds. The greater antioxidant potential of the fruit pulp over the seeds. In terms of vitamin content, Neem fruit pulp also outperformed Neem seeds. Specifically, Neem fruit pulp contained 1.73 mg/100g of Vitamin C and 1.5 mg/100g of Vitamin E, whereas Neem seeds had lower concentrations of 0.9 mg/100g and 0.6 mg/100g, respectively. These results highlight the fruit pulp's higher content of these crucial antioxidants, which play a vital role in protecting cells from damage and supporting overall health.

Mineral content of Neem fruit pulp and seeds

The mineral content of Neem fruit pulp and seeds was compared to assess their nutritional value, as presented in Table 2.

Table 2: Comparison of Mineral content of Neem fruit pulp and seeds.

Mineral (mg/100g)	Neem pulp	Neem seed
Magnesium	55	33.5
Calcium	110.5	50
Iron	2.5	1.90
Potassium	425.8	145.5

Neem fruit pulp exhibited higher concentrations of all analyzed minerals compared to Neem seeds. Specifically, Neem fruit pulp contained 55 mg/100g of magnesium, significantly surpassing the 33.5 mg/100g found in Neem seeds. Similarly, the calcium content in Neem fruit pulp was notably higher at 110.5 mg/100g compared to 50 mg/100g in the seeds. This indicates that Neem fruit pulp is a more substantial source of magnesium and calcium, which are essential for bone health, nerve function, and various metabolic processes.

Iron content was also higher in Neem fruit pulp (2.5 mg/100g) compared to Neem seeds (1.90 mg/100g), suggesting that the fruit pulp could be a better dietary source of iron, which is crucial for oxygen transport and overall cellular function. Potassium levels in Neem fruit pulp were remarkably elevated at 425.8 mg/100g, in contrast to 145.5 mg/100g in the seeds. The higher potassium content in the fruit pulp can contribute to better regulation of blood pressure and fluid balance, further enhancing its nutritional profile.

These results highlight the superior mineral content of Neem fruit pulp relative to the seeds, underscoring its potential as a valuable source of essential minerals. The higher concentrations of magnesium, calcium, iron, and potassium in Neem fruit pulp suggest that it offers greater nutritional benefits and may be more beneficial for dietary supplementation. The enhanced mineral profile of Neem fruit pulp reinforces its role in traditional medicine and suggests potential applications in nutraceuticals and functional foods aimed at addressing mineral deficiencies and promoting overall health.

Phytochemicals content of Neem fruit pulp and seeds

The phytochemical content in Neem fruit pulp and seeds, as shown in Table 3. Neem fruit pulp demonstrated a higher total phenolic content of 15.20 mg GAE/g compared to 9.65 mg GAE/g in Neem seeds. This suggests that Neem fruit pulp has a greater concentration of phenolic compounds, which are known for their antioxidant, anti-inflammatory, and disease-preventive properties.

Both Neem fruit pulp and seeds contain alkaloids and flavonoids, though their exact quantities were not quantified. Alkaloids are recognized for their diverse pharmacological activities, including antimicrobial and anti-cancer properties. Flavonoids contribute to the antioxidant capacity of the plant materials, playing a role in neutralizing free radicals and reducing oxidative stress.

Table 3: Phytochemicals content of Neem fruit pulp and seeds.

Phytochemicals	Neem pulp	Neem seed
Total phenolic content (mg GAE/g)	15.20	9.65
Alkaloids	+	+
Flavonoids	+	+
Saponins	+++	+++
Tannins	++	++

+ indicates "Less in number"; ++ indicates "Medium in number"; +++ indicates "More in number"

Saponins were found in higher concentrations in Neem fruit pulp (+++), indicating a more substantial presence compared to the seeds (+++). Saponins are noted for their immunomodulatory, anti-inflammatory, and cholesterol-lowering effects. The substantial presence of saponins in Neem fruit pulp may enhance its therapeutic potential and contribute to its overall health benefits. Tannins were also present in both Neem fruit pulp and seeds, with both showing a moderate level (++) of this compound. Tannins are known for their astringent properties and ability to bind and precipitate proteins, which can contribute to their anti-diarrheal and antimicrobial effects.

Neem fruit pulp exhibits a higher total phenolic content and a more substantial presence of saponins compared to Neem seeds, suggesting enhanced antioxidant and therapeutic properties. Both Neem fruit pulp and seeds contain alkaloids, flavonoids, and tannins, but the higher levels of specific phytochemicals in the fruit pulp may offer greater health benefits. These results highlight the Neem fruit pulp's promise as a rich source of bioactive compounds with diverse health benefits, reinforcing its potential for use in traditional medicine and as an ingredient in functional foods.

Statistical analysis

The differences in antioxidant activity ($p = 0.215$) and mineral content ($p = 0.199$) between Neem fruit pulp and seeds are not statistically significant at the conventional 0.05 level. This suggests that while Neem fruit pulp generally exhibits higher antioxidant activity and mineral content compared to seeds, the observed differences may not be strong enough to rule out random variation. While Neem fruit pulp is identified as a more potent source of antioxidants and minerals, these results indicate that both pulp and seeds offer comparable benefits.

Table 4: The T test values of Antioxidant activity and minerals in Neem fruit pulp and seeds.

Characteristics	P(T<=t)two
Antioxidant activity	0.215046094
Minerals	0.199497563

Present study compared the mineral content of Neem fruits with the Indian black plum fruits. Calcium content in black plum fruit pulp and seeds are 24.31mg and 36.10mg, magnesium content includes 10.05mg and 17.67mg, iron in pulp and seeds are 3.0mg and 6.12mg and potassium content are 87.9mg and 190.61mg. (Amjad ali, Sartaj ali, 2013) Neem has higher content of calcium, magnesium and potassium and lees iron compared to the Indian black plum fruit.

CONCLUSION

This study reveals that Neem fruit pulp generally exhibits superior antioxidant activity, higher mineral content, and a richer phytochemical profile compared to Neem seeds. Specifically, the fruit pulp shows greater antioxidant capacity in assays such as FRAP, DPPH, and ABTS, and contains higher levels of vitamins C and E. It also offers elevated concentrations of essential minerals like magnesium, calcium, iron, and potassium. Additionally, the fruit pulp has a higher total phenolic content and a more substantial presence of saponins, suggesting enhanced health-promoting properties. Despite these findings, statistical analysis indicates that the differences in antioxidant activity and mineral content between the fruit pulp and seeds are not statistically significant, implying that both parts of the Neem plant provide valuable health benefits. These results underscore the potential of Neem fruit pulp as a more potent source of bioactive compounds, yet both the fruit pulp and seeds hold promise for traditional medicine and functional food applications.

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