

MEDICINAL ASSESSMENT OF KENAF *(Hibiscus cannabinus L.)***: ANTIBACTERIAL, PHYTOCHEMICAL, AND NUTRITIONAL PROFILING**

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ABSTRACT

While pharmaceutical drugs often come with detrimental side effects such as liver damage and addiction, the *Hibiscus cannabinus L.* (kenaf) plant has proven to be a promising traditional medicinal alternative. However, there are extremely few studies to investigate the variability of medicinal and nutritional parameters in Kenaf tissues. The phytochemicals, proximate composition, mineral content, amino acid content, and antibacterial activity of kenaf tissues have been studied and compared using advanced techniques. UPLC analysis reveals that leaves contain the highest concentration of caffeic acid (76.4 mg/100 g), which is also present in stem bark (51.3 mg/100 g). GC-MS analysis shows linoleic acid is predominant in seeds (46.23%) and stem bark (40.7%), while E-Phytol is mostly in leaves (33.5%) and hexadecanoic acid in flowers. Phenolic analysis indicates water as the most effective extraction solvent, with leaves showing the highest phenolic content (592.1 mg/100 g). Water remains the best solvent for flavonoid extraction, with flowers and leaves having the highest flavonoid concentrations (722.1 mg/100 g and 552.2 mg/100 g, respectively). Seed is the most nutritious, containing the highest amount of crude protein and nitrogen-free extract. Stem bark is rich in calcium (883 mg/kg) and potassium (3093 mg/kg). Amino acid analysis shows seeds are high in glutamic acid and aspartic acid, while proline is predominant in stem bark. Leaf tissue exhibits the strongest antibacterial activity against *E. coli and S. aureus*, suggesting its potential use in antibacterial applications. This demonstrates the research's contributions to possible uses of Kenaf in health and nutrition.

Keywords: Profiling, Phytochemicals, Nutritional Analysis, *Hibiscus cannabinus L.*, Antibacterial

INTRODUCTION

Hibiscus cannabinus L. is a herbaceous and dicotyledonous plant native to Africa. It is widely distributed due to its ability to flourish in a variety of temperatures (Kandi & Charles, 2018). In the past, kenaf has been grown for its use in cordage, paper, and textiles. The plant's leave was reported to possess so many medicinal properties including hepatoprotective, aphrodisiac, anti-inflammatory, antioxidant, and anticancer activities (Gabriel *et al.,* 2005; Ryu *et al.,* 2013; and Sim & Nyam, 2021). Kenaf leaves are used to treat Guinea worm illness and anemia in traditional African medicine (Ayadi *et al.,* 2016). Furthermore, they have been reported to be helpful in Ayurvedic treatment for ailments like diabetes, biliousness, blood problems, weariness, and throat infections (Ryu *et al.,* 2017; Pascoal *et al.,* 2015). Kenaf extract has also been shown to stimulate the stomach and improve its function, as well as help treat scurvy and jaundice (Pascoal *et al.,* 2015). It has usage in the food and pharmaceutical industries as a valueadded product, in addition to its therapeutic applications (Maganha *et al.,* 2010; Nandagopalan, *et al.,* 2015; and Monti & Alexopoulou, 2013). They are also used as useful ingredients in bread goods and in healthy beverage formulations (Lim *et al.,* 2020). It is also recognized as a nutritional supplement for tea prepared from kenaf leaves (Maganha *et al.,* 2010).

Bioactive substances called phytochemicals are present in plants and offer a number of health advantages (Mustapha *et al.,* 2023). Advanced analytical techniques such as UltraPerformance Liquid Chromatography (UPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) are employed to detect and quantify these substances. These methods give a thorough analysis of the phytochemicals found in the various Kenaf tissues, providing information on their possible health advantages and commercial uses (Garcia-Salas *et al.,* 2010; Mubarok *et al.,* 2021).

A nutritional study is necessary to comprehend Kenaf's dietary value. In order to provide a thorough profile of the plant's nutritional content, this involves figuring out the moisture content, crude protein, crude fiber, crude fat, ash, and nitrogen-free extract (Chukwu & Onuh, 2020). Furthermore, mineral analysis aids in determining the concentrations of vital minerals in Kenaf tissues, including calcium (Ca), phosphorus (P), potassium (K), zinc (Zn), and iron (Fe). These minerals enhance the plant's overall nutritional quality and are essential for a number of body processes (Ullah, 2013). Since amino acids are the building blocks of proteins and are essential for many physiological functions, the amino acid composition of Kenaf is also of interest. Analyzing the amino acid composition of Kenaf aids in assessing the protein's purity and its health advantages (Emerue *et al*., 2022). *Staphylococcus aureus* and *Escherichia coli* are known to cause a variety of infections, and because antibiotic resistance is becoming a bigger problem, natural antibacterial agents are being sought after as alternatives to synthetic antibiotics (Borges et al., 2016; Ríos & Recio, 2005).

MATERIALS AND METHODS Plant Materials and Extraction

The cultivars of "Baekma" were examined. The kenaf plant's leaves, stem bark, flowers, and seeds were collected and airdried. Each sample (7 g) was extracted using or eight hours using 75 milliliters of methanol, water, ethanol, and chloroform. Samples that are extracted are used for Ultra-high Performance Liquid Chromatography (UPLC), and total polyphenol content and flavonoid content were determined.

Ultra-high Performance Liquid Chromatography (UPLC)

A UPLC system (CBM-20A, Shimadzu) with two gradient pumps (LC-30AD, Shimadzu), a UV detector (SPD-M30A, Shimadzu), an auto sample injector (SIL-30AC, Shimadzu), and a column oven (CTO-30A, Shimadzu) at Mewar University were used to evaluate functional chemicals . An XR-ODS column $(3.0 \times 100 \text{ mm}, 1.8 \text{ µm}, \text{Shimadzu})$ was used for the separation, and solvents A and B (0.1% trifluoroacetic acid in distilled deionized water and acetonitrile, respectively) were used in a linear gradient elution. A 0.45 micrometer membrane was used to filter the ground samples (4.5 g) after they were extracted for 60 minutes in 4.5 mL of water.

Gas Chromatography Mass Spectrometry (GC-MS)

Plant extraction for GC-MS analysis was done at Sangam University using a modified version of Ryu *et al.* (2013) method. After two hours of extracting 8 grams of powdered leaf, stem bark, flower, and seed in 45 mL hexane, 500 µL of 2 N potassium hydroxide in methanol was added. Two milliliters of extracts from various portions of the kenaf plant were subjected to analysis using a Shimadzu GC-MS equipped with an HP-88 capillary column (60 m \times 0.25 mm \times 0.25 µm, J&W Scientific). With an ionization voltage of 70 eV, a mass scan range of 50–450 mass units, 230 degree celsius injector and detector temperatures, a one microliter injection volume, a 1:30 split ratio, and a 1.6 mL/min flow rate of helium carrier gas, the setup is as follows. The temperature of the column was raised to 180 degrees Celsius at five Celsius per minute, 280°C at 1°C/min, and 40°C for five minutes. Retention time and the NIST 62 Library mass spectra database were used to identify the substances.

Phenolic Content Analysis

The Folin-Ciocalteau colorimetric technique was used with slight modification to determine the total phenolic content (TPC) (Jin *et al.,* 2013). One and a half milliliters (0.2 mL) of 20% Folin-Ciocalteau reagent were combined with them. The mixture was left at room temperature for ninety minutes in the dark after four milliliters of 7% Na₂CO₃ was added and diluted to ten milliliters with water. Absorbance was then determined with a UV spectrophotometer (UV-1800, Shimadzu) at 760 nm. Tannic acid curve calibration was used to calculate TPC.

Flavonoid Content Analysis

Zhishen *et al.* (1999) approach was used with modification to calculate the total flavonoid content (TFC) in different part of kenaf. Each extract (0.3 mL) was combined with 0.3 mL of 5% NaNO² and 5 mL of double-distilled water. 0.4 mL of 10% AlCl³ was added after 5 minutes. After 6 minutes, 3 mL of NaOH was added, and water that had been double-distilled was used to dilute the mixture to 10 mL. At 510 nm, absorbance was measured. A curve of calibration of quercetin equivalents was used to determine TFC.

Proximate Analysis

The standard analytical procedures (Horwitz *et al.,* 1975) were used to assess the following food quality parameters: moisture, ash, crude protein, crude fiber, crude fat, and nitrogen-free extract contents.

Mineral Content Analysis

The kenaf's mineral contents were ascertained through the application of modified analytical techniques derived from (Horwitz *et al.,* 1975). Samples (2g) were burned at 500 °C in a muffle furnace using a clean porcelain crucible and dry ashing. The resulting ash was then heated gently on a heating mantle until the brown fumes subsided. It was then dissolved in 5.0 mL of an HNO3/HCl/H2O mixture (1:2:3). Each sample was then mixed with 6.0 mL of distilled water and boiled until a colorless solution formed. After that, the mineral solution was filtered into a 100 mL volumetric flask and its elemental composition was examined in triplicate using an atomic absorption spectrophotometer (Thermo Scientific™ iCE 3000 Series).

Amino acid Content

The amino acid analyzer (Shimadzu AAA-Direct) was utilized at Mewar University to ascertain the amino acid contents. The samples were hydrolyzed for 23 hours at 120 $\rm{^{\circ}C}$ in a N₂ environment using hydrochloric acid (6 mol/dm³). Following filtration, all hydrolysates were preserved at 4°C for 24 hours before being neutralized with a sodium hydroxide solution and normalized to a volume of 100 mL using sodium citrate buffer (pH 2.2). Sample hydrolysates were employed in a particular reaction after undergoing a chromatographic separation in sodium citrate buffer.

Antibacterial Activity

Disc diffusion was used to measure the antibacterial activity (Adnan *et al*., 2020). The microorganisms employed were Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. The strains were grown for one day at 45 °C on a Nutrient Broth medium. A sterile filter paper disk with a diameter of 6 mm was placed on top of the 15 mL of nutrient agar medium that had been poured into petri dishes using laminar airflow and allowed to settle. Following this, 100 µL of bacterial suspension (107 CFU/mL) was scattered across the surface of the agar plates. Moreover, the discs were loaded with 50 mg/mL of each extract and incubated for 24 hours at 37 °C. The inhibitory zones' diameter was measured and reported in mm. As a negative control, 5% dimethyl sulfoxide was utilized (Cesonien *et al.,* 2021).

RESULTS AND DISCUSSION

Ultra-Performance Liquid Chromatography (UPLC)

Figure 1 displays the representative UPLC fingerprint chromatograms of the various kenaf plant sections. Numerous phytochemical compounds present in the stem bark, seeds, flowers, and leaves of the Kenaf plant, as well as their concentrations were reported. Caffeic acid is present in leaves at the highest concentration (76.4 mg/100 g), but it is also present in stem bark (51.3 mg/100 g), flowers (17.7 mg/100 g), and seeds (none). Kaempferol glycoside (59.2 mg/100 g) and afzelin (38.5 mg/100 g) were found in the leaves. They were also present in the stem berk and absent in the other tissues. With the exception of the leaves, all components contain p-hydroxybenzoic acid, with seeds having the highest concentration (95.5 mg/100 g). Vanillin was not present in flowers or leaves. However, it is present in seeds (44.3 mg/100 g) and stem bark (19.9 mg/100 g). Isoquercitrin was not present in flowers or leaves, however it is present in the

stem bark $(9.5 \text{ mg}/100 \text{ g})$ and seeds $(18.0 \text{ mg}/100 \text{ g})$. It is crucial to note that p-hydroxybenzoic acid predominates in seeds and flowers. P-hydroxybenzoic acid exhibits high recoveries in urine and it has no detectable drug concentration in kidneys, making it an efficient preservative in pharmaceuticals, tablets, and human body fluids (Jones *et al.,* 1956). The phytochemical distribution of the Kenaf plant varies depending on the part of the plant; the leaves are especially rich in a variety of chemicals, as seen in figure 1.

Gas Chromatography-Mass Spectrometry (GC-MS)

A potent analytical method for separating and measuring volatile chemicals in a sample is gas chromatography-mass spectrometry (GC-MS) (Skog *et al.,* 2019). Retention times and total percentages offer important details regarding the makeup and characteristics of the substances under study. The retention time, which is a measure of how long a compound stays in a chromatographic column (Prodhan *et al.,* 2018), varies from 14.1 to 19.68 minutes for each component. Due to the various chemical characteristics of each compound, there are variations in the way that each one interacts with the material in the column.

Table 1: Compounds Present in Kenaf Seed

The substances identified by GC-MS as being present in the kenaf seed are presented in Table 1. The greatest overall amount (46.23%) was found in 9,12-octadecadienoic acid, sometimes referred to as linoleic acid, demonstrating its abundance. This substance most likely has a major function in Kenaf seeds. It has a significant nutritional value because of its effects on cellular activities, metabolism, and overall health (Chen *et al.,* 2021). Furthermore, there is a significant

amount of 9-Octadecenoic acid (cis) (27.38%). Since they are unsaturated fatty acids, both of these acids may enhance the nutritional value of the seed (Mæhre *et al.,* 2018). Conversely, the smaller amounts of 9-Hexadecenoic acid and cis-10- Heptadecenoic acid (3.2% and 0.99%, respectively) indicate their negligible occurrence. In the middle are 9- Octadecadienoic acid (trans) (6.8%) and octadecanoic acid (15.5%) .

inhibitory concentration of 2 micrograms/ml (Islam *et al.,* 2018). Another important component, hexadecanoic acid, makes up a sizable amount at 21.8% and has a retention time of 15.8 minutes. Despite having different retention durations of 14.4 and 19.4 minutes, respectively, compounds such as 2- Pentadecanone, 6,10,14-trimethyl, and 9,12-Octadecadienoic acid similarly significantly contributed to the composition at 14.4% and 15.6%. Despite having comparatively longer retention durations of 18.2 and 18.76 minutes. Other molecules such Phytol acetate and 9-Octadecenoic acid contribute 6.9% and 9.1%, respectively, suggesting a moderate abundance.

The substances that the GC-MS detected in flower tissue are listed in Table 3. Notably, the Retention Times (RTs) of hexadecanoic acid are the shortest at 15.3 minutes. With a dominance of 30.1%, hexadecanoic acid is followed by 9 octadecenoic acid (24.5%). The fact that 15methylhexadecanoic acid makes up only 3.0% of the sample suggests that it is barely present. Hexadecanoic acid, which dominates, is an anti-inflammatory substance that inhibits phospholipase A2, supporting Ayurveda's use of it to treat rheumatic complaints (Chen *et al.* 2021).

Table 4 shows that, when taking into account the kenaf stem bark, the RT for 9-hexadecenoic acid is 15.9 minutes, the RT for octadecanoic acid is 17.5 minutes, and the RT for 9 octadecenoic acid is 18.2 minutes. These RT values shed light on how they behaved when they were apart. Similar to seed,

octadecanoic acid (20.1%) and 9,12-octadecadienoic acid (40.7%) are the two most notable components. There are lesser levels of 9-hexadecenoic acid (20%) and phytol acetate (7.2%) .

Total Phenolic Content Table 5: Phenolic Content

Flavonoids (Kho *et al.,* 2019) and phenolic compounds (Pascoal *et al.,* 2015) are thought to be responsible for the medicinal effects of kenaf. These chemicals have been shown to have antibacterial and antioxidant properties (Nimse & Pal, 2015; Kandi & Charles, 2018; Ma'aruf *et al.,* 2024). The phenolic content of plant components extracted using water, methanol, ethanol, and chloroform is shown in Table 5. These parts include the seed, flower, leaf, and stem bark. According to the findings, water was the best solvent for phenolics extraction, particularly leaves, which have the maximum content of phenolics (592.1 mg/100 g). At 310.6 mg/100 g,

flowers also show a high phenolic content in water. Though less than water, methanol extracts large amounts of phenolic compounds from flowers and leaves (294.7 mg/100 g and 211.9 mg/100 g, respectively). Ethanol extracted less phenolic content than methanol and water, especially from leaves and flowers. Conversely, chloroform extracted the least amount of phenolics compared with other solvents particularly low quantity observed in flowers and stem bark. This highlights the greater performance of water over chloroform as a solvent for phenolic extraction, especially from the leaves.

Table 6 shows the flavonoid concentration of several the plant parts that were extracted using water, methanol, ethanol, and chloroform, including the seed, flower, leaf, and stem bark. Flowers and leaves, had flavonoid concentrations of 722.1 mg/100 g and 552.2 mg/100 g, respectively. water was the most efficient solvent used. Although less than water, methanol also removes a sizable amount of flavonoids, particularly from flowers (650.7 mg/100 g). The effectiveness of ethanol is significantly lower, and all plant parts have a noticeably lower level of flavonoid concentration. The least efficient extraction solvent was chloroform, which extracted very little flavonoids in all parts of the plant of the plant. This result demonstrates how effective water is in removing flavonoids, especially from flowers and leaves.

Proximate Analysis

Figure 2 displays the results of a proximate analysis conducted on the different parts of the Kenaf plant. The parameters that were assessed in this study were moisture, ash, nitrogen-free extract, crude protein, crude fiber, and crude fat . The flower has the highest (25.8 mg/kg) and lowest (7.9 mg/kg) moisture content than the seeds. The Seeds are regarded as being extremely nutritious due to their high crude protein content (26.4 mg/kg) and high crude fat content (21.4 mg/kg).

Figure 2: Result of Proximate Analysis of Kenaf Tissues (mg/kg)

Conversely, stem bark has the highest crude fiber content (38.3 mg/kg), indicating potential dietary fiber benefits. Because of its high crude protein content (23.5 mg/kg) and nitrogen-free extract content (39.4 mg/kg), the leaf has a high energy value. The nitrogen-free extract concentration (54.64 mg/kg), a proxy for the amount of non-fiber carbohydrates, is highest in the flower.

Mineral Content of Kenaf Tissues

Figure 3 displays mineral composition of Kenaf parts and illustrates the significant diversity in the distribution of calcium (Ca), phosphorous (P), potassium (K), zinc (Zn), and iron (Fe) in the various plant parts. Because the stem bark has a remarkably high calcium content (883 mg/kg) and potassium content (3093 mg/kg), it could be an excellent source of these essential minerals. Adequate consumption of calcium has demonstrated health advantages, including a decrease in pregnancy-related hypertensive diseases, a reduction in blood pressure, a prevention of osteoporosis, and a reduction in cholesterol levels (Cormick & Belizán, 2019).

Figure 3: Mineral Content of Kenaf Tissues

According to He and MacGregor (2008), potassium is also necessary for humans since it lowers blood pressure, lowers the mortality rate from cardiovascular disease, and lowers the excretion of calcium in the urine, which may lessen the risk of osteoporosis. The Seeds contain the highest concentration of phosphorus (803 mg/kg), along with significant amounts of potassium (1450 mg/kg) and iron (56 mg/kg). With significant quantities of iron (58.3 mg/kg), potassium (2190 mg/kg), and phosphorus (464 mg/kg), flowers display a balanced mineral composition. All the elements that have been analyzed are found in leaves in moderate concentrations; potassium was the most concentrated of the minerals found among the plant parts (1394 mg/kg); while zinc and calcium were found in substantial concentrations, at 189.9 mg/kg and 179 mg/kg, respectively.

Table 7: Amino Acid Composition

Table 7, describes the amino acid composition of Kenaf different parts. It shows differences in concentrations of various amino acids in stem bark, seeds, flowers, and leaves. The highest concentrations of various amino acids, particularly glutamic acid (166.97 mg/kg) and aspartic acid (132.34 mg/kg), were found in the seeds, and these indicate a rich protein profile. The stem bark contains considerable amounts of proline (153.10 mg/kg), aspartic acid (13.88 mg/kg), and histidine (12.42 mg/kg). Flowers have a high quantity of leucine (19.75 mg/kg), but moderate levels of proline (27.34 mg/kg) and serine (23.69 mg/kg). Proline (414.54 mg/kg) and cystathionine (13.79 mg/kg) are among the noteworthy amounts of amino acids found in the leaves, along with tyrosine (6.49 mg/kg) and serine (15.46 mg/kg). According to research, seeds contain a large amount of glutamic acid, which helps cancer patients live better lives by reducing symptoms and preventing mucosal damage (Anderson & Lalla, 2020). Seeds also contain a lot of aspartic acid. It is linked to neurogenesis, the endocrine system, protein production, and neurotransmission (Anderson & Lalla, 2020). According to Krishnan *et al.* (2008), proline, which predominates in stem bark, increases cell lifespan by shielding cells from oxidative stress.

Table 8: **Antibacterial Activity**

Table 8 displays the zone of inhibition, measured in millimeters of the bacteria by the components of the different plant's parts. This illustrates the antibacterial efficiency of the different plant parts varies noticeably. The leaves exhibit the strongest antibacterial action against E. coli (11.37 mm) and S. aureus (12.83 mm). The stem bark also shows good activity, with inhibitory zones of 9.14 mm against E. coli and 9.65 mm against S. aureus. The flower tissue shows moderate antibacterial activity against S. aureus (10.44 mm), especially when compared to E. coli (6.03 mm). The seeds exhibited the least antibacterial activity; its zones of inhibition for E. coli and S. aureus are 7.48 mm and 8.13 mm, respectively. The part that is generally most effective against both bacteria is leaf suggesting that the leaves may be used in antibacterial applications.

CONCLUSION

This study uses advanced analytical methods and a lot of biochemical tests to look closely at the phytochemical, nutritional, and antibacterial properties of kenaf (*Hibiscus cannabinus L*.). We study here the phytochemicals, flavonoids, phenolic compounds, amino acids, mineral contents, and antibacterial activity across different Kenaf tissues. In a novel way, this work investigates the intricate relationships between these parameters in a variety of Kenaf tissues with comparison. The leaves, which contain the highest levels of flavonoids and caffeic acid, show strong antibacterial activity against S. aureus and E. coli, indicating that they may find utility in antibacterial applications. With the highest concentrations of pure protein and nitrogen-free extract, as well as important amino acids like glutamic and aspartic acid, seeds are extremely nutrient-dense. Important elements like calcium and potassium are abundant in stem bark, which adds to its nutritional advantages. These results highlight the diverse potential of kenaf tissues for nutrition and health, highlighting their nutritional and medicinal worth as well as prospective uses in the creation of functional foods and natural remedies.

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