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EVALUATING PHARMACOGNOSTIC PARAMETERS AND MUTAGENIC ACTIVITY OF *KHAYA SENEGALENSIS* (DESR.) A. JUSS.

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Keywords:

Khaya senegalensis, Raw material, Quality control, Simple methods

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ABSTRACT: The stem bark of *Khaya senegalensis*, known as mahogany is commonly sold on the Ghanaian market for the treatment of numerous ailments. To ensure its safe and efficacious use, there is a need to ensure that this plant material is of the best quality. Quality will involve ensuring its identity, purity and content are up to recommended standards by applying available and relevant methods. As a means to improve the safe use of the material, mutagenic studies were also conducted. This study sought to evaluate parameters that can be easily applied to evaluate the quality of the stem bark of *K. senegalensis*. Macroscopy, microscopy, physicochemical, and phytochemical investigations using fluorescence and UV analysis were employed. Microscopic analysis of *K. senegalensis* leaf revealed the presence of calcium oxalate crystals, trichomes, stomata cells, and vein islets. Phytochemical screening of the stem bark showed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, and terpenes. UV analysis displayed spectra that shift and change in absorbance on acidification and basification of the aqueous and ethanol extracts. Under UV light of short and long wavelengths, changes in fluorescing of the extracts were observed in various solvents. The characteristic macroscopic, microscopic, physicochemical, and chemical parameters evaluated for *K. senegalensis* can be employed in the simple authentication and establishment of the quality of raw materials of this plant. This will improve the quality and hence efficacy of *K. senegalensis* when used for treating diseases and manufacturing medicines.

INTRODUCTION: Herbal remedies are used worldwide for both minor and serious ailments [1-3]. Due to poverty and limited access to contemporary medicine, about 80% of the world's population is believed to use herbal remedies as their source of primary healthcare [4-6].

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Herbal medicines are in great demand, especially in developing countries such as Ghana, for primary healthcare. This is because of their efficacy, safety, low cost, and lesser side effects. However, the non-availability of pharmacopoeial standards with its requisite analytical equipment for plant-derived medicines is a major drawback that frustrates clinical trials and thus overshadows the time tested healing properties of plant medicines [7].

In this respect, *K. senegalensis*, which is one of the medicinal plant sold in high quantities on the Ghanaian market [8, 9] and is also a component of many Pharmacopoeial medicinal preparations of

multiple African groups [10], was evaluated for its quality control parameters. This will ensure the quality of the raw plant materials in a setting such as Ghana.

Khaya senegalensis (Desr.) A. Juss is of the family Meliaceae. It is commonly known as African mahogany. It is an evergreen tree that grows up to a height of 18 m high, up to 1 m in diameter, with a clean bole, 6-9 m long. Buttresses are not conspicuous or absent. It is cultivated as avenue trees in many towns and cities [11]. This plant is commonly formulated as decoctions and tinctures. *K. senegalensis* is used extensively in West Africa as a bitter tonic, febrifuge, blood tonic and antimalarial [8]. The extract from the bark has analgesic, anticonvulsant, anthelmintic (human and veterinary), antimicrobial, antipyretic, hematinin; febrifuge, sedative and emetic properties. It is used in the treatment of jaundice, scorpion bites, allergies, dermatoses, infections of the gums, wounds, constipation, chronic weakness, headache, heat rash, loss of appetite, malaria, ulcer, and sexually transmitted disease [11]. It is known to be a potential arbortifacient [12, 13].

The antipyretic, anticonvulsive, analgesic, and sedative properties are believed to be due to the simple coumarins (*e.g.*, scopoletins) present in the stem bark. Aqueous extracts of the stem bark and leaves containing limonoid have also demonstrated a stronger antisickling activity [13, 14]. In addition to the bark, both the seeds and leaves have also been used as medicines for treating fever and headache, whilst preparations made from the roots have been used against syphilis, leprosy and as an aphrodisiac [15].

On the other hand, this plant has also been shown to exhibit some toxicity. A study indicates that the aqueous stem bark extract of *K. senegalensis* may affect the cellular integrity of vital organs of the body [11, 16]. Renal toxicological assessments of sulphonated nanocellulose isolated from *Khaya sengalensis* seed has also been shown to potentially toxic in rats [17].

Various parts of this plant have various other non-medicinal uses such as fodder, fuel, fiber, timber, gum and resin dyestuff, oils and even as fish poison [11]. The bark can be used for tanning leather as it is known to contain high quantities of tannins [18, 19].

Also, the current widespread increase in the use of herbal medicines have arisen with it immense safety concerns. For example, the consistent increase in cancer cases in Ghana has led to the advancement of various theories with regards to the causes. Among the possible proposed causes are commonly used substances, which may include some herbal medicines. This notion may not be totally wrong since some medicinal plant products have been proven to contain components that are potential mutagens [20-24]. It is also important to note that most of such traditionally used medicinal plants have not been subjected to exhaustive toxicological investigations. Only a small proportion of such plants have been thoroughly investigated scientifically for their acclaimed benefits and side effects [25]. Reports have it that a number of such plants used as food or in traditional medicines may exhibit *in-vitro* mutagenicity [26-28] and 90% of such mutagens could be carcinogens capable of inducing cells to undergo abnormal growth and genetic defects [28, 29], hence the rational to perform the mutagenic test on such a widely used plant. To screen for mutagenicity, the Ames test which is very commonly employed because it enables the screening of many chemicals rapidly and inexpensively was used [30, 31]. If found to be mutagenic, this can be further investigated in animal model [32].

MATERIALS AND METHODS:

Plant Material: The leaves and stem barks of *K. senegalensis* were collected from the botanical garden of the University of Ghana. The samples were authenticated at the Ghana Herbarium, Department of Plant and Environmental Biology, University of Ghana. A voucher specimen (number PSM68/19) have been kept in the herbarium of the Department of Pharmacognosy and Herbal Medicine, University of Ghana, Legon. Fresh leaves were obtained, washed with water, and stored in glycerin to be later used for microscopic analysis. The stem bark was air-dried for three weeks and milled into coarse powder, and kept in well-labeled air-tight containers for further analysis.

Macroscopic Evaluation: The dried stem bark of *K. senegalensis* was preliminarily analyzed for color, odor, texture, and fracture, while leaves were analyzed for their shape, color, arrangement, apex base, texture, margin, and venation.

Microscopic Evaluation: The microscopic characteristics of the leaves were studied with a Leciad compound light microscope using procedures recommended by WHO guidelines on Quality control methods for herbal materials, 2011 [33]. Four-millimeter square (4 mm^2) sizes of the mature lamina of the leaves were cut with a microtome. The lamina was then boiled for about two hours in chloral hydrate solution to clear the chlorophyll content. The sections were mounted on a clean glass slide with the help of glycerin and covered with a coverslip. The slides were observed under a light microscope at an objective magnification of x10. The presence of calcium oxalate crystals, stomata, and trichomes was observed. Quantitative parameters such as vein islet number, veinlet termination number, stomata number, and stomatal index were calculated according to standard protocols as described by WHO, 2011.

Powdered Microscopy: Small quantities of milled stem bark powder were mounted in 2-3 drops of chloral hydrate on a glass slide. Each slide was covered with a coverslip and examined under the microscope. The samples were photographed for lignified fibers, calcium oxalate crystals, and stomatal cells. For the observation of lignified tissues, a small quantity of the stem bark was mounted in phloroglucinol and concentrated HCl and observed under low power. Brachysclereids, which were stained pink to purple, were identified and also photographed.

Physico-chemical Analysis: Total ash, acid insoluble, and water-soluble ash values were estimated, and extractive values of petroleum ether, 50 % ethanol, and water were also determined for the air-dried stem bark. Furthermore, the foreign organic matter, swelling index, and the foaming index were analyzed according to methods described by WHO, 2011 [33].

Preliminary Phytochemical Screening: Preliminary phytochemical screening was performed to detect the presence of alkaloids, saponins, tannins, flavonoids, glycosides, and terpenes as per the methods of Khandelwal, 2002 and Harbourne, 1992 [34, 35].

Fluorescence Studies: Characteristic fluorescence of the powdered stem bark of *K. senegalensis* was carried out in distilled water, dilute NaOH, dilute HCl, methanol, ethanol as well as the respective acidified and basified solvents. This was performed according to methods described in published literature [36]. Observations of the samples were made under visible daylight and UV light of short wavelength (λ254 nm) and UV light of long-wavelength (365 nm) for their characteristic colors [37].

Ultraviolet/Visible Spectrometric Analysis: An aqueous extract was prepared by weighing 10 g of coarsely powdered air-dried stem bark of *K. senegalensis* into 100 mL of distilled water. The plant material were sonicated three times for intervals of 15 min each. The solution was filtered into a round-bottomed flask and concentrated using a rotary evaporator set at 45 ºC. The residue was freeze-dried to obtain the crude aqueous extract. An amount of 20 mg of this extract was accurately weighed and separately dissolved in 20 mL of distilled water, dilute hydrochloric acid (0.1M), dilute sodium hydroxide (0.1M), methanol, acidified methanol, and basified methanol. Each of these preparations was sonicated for 10 min to ensure complete dissolution of the extracts. Each of the sonicated solutions was allowed to cool to room temperature and filtered using membrane filter 0.45μm. Dilutions of 1 in 10 of each of the solution were made in the respective solvents. The diluted solutions were filtered and analyzed using a 1 cm quartz cell/cuvette (sample holder). All the samples were scanned over a wavelength range of 200 – 600 nm (Ultraviolet/ visible range) using a single beam Jenway 7315 UV- Vis spectrophotometer.

Heavy Metal Analysis: An Olympus Vanta Portable ED-XRF (VMR) analyzer equipped with 4-Watt X-ray tube with application-optimized anode material rhodium (Rh), tungsten (W), 50kV x-ray tube, and large area silicon drift detector was used to analyze the sample for heavy metals. Calibration of the XRF was done using the SARM 2711A, certified reference material from the manufacturer. Twelve (12 g) grams of the stem bark powder was sieved with a sieve of 180 μm mesh size into a fine powder and kept in a dry well-labeled container for analysis. The loose sample was irradiated following the manufacturer's protocol, and simultaneous measurement of the levels of heavy metals present was performed.

Microbiological Analysis:

Bacterial and Fungal Counts: One gram (1 g) of the powdered stem bark of *K. sengalensis* was weighed into 100 mL of sterile water in a tube to make a stock concentration. The tubes for the dilution were labeled as: a (10^2), b (10^4), c (10^6), d (10^8), e (10^{10}). Using a micropipette, 1 mL of the content of tube (a) was transferred to the tube (b) containing 99 mL of sterile water and mixed gently. The process was repeated until all the tubes containing 99 mL of sterile water were mixed. An aliquot of 1 mL of each content was taken and added to their corresponding tubes of molten nutrient agar at 45 ºC. Each tube was poured into its labeled corresponding sterile petri dish (A, B, C, D, and E). The petri dishes were inverted and incubated at 37 ºC for 48 h and the number of colonies counted. A similar procedure was employed in estimating the fungal count in the sample, but instead, potato dextrose agar was used. The petri dishes were incubated upright at room temperature for 5 days. *Bacillus subtilis* ATCC 6538-P and *Candida albicans* ATCC 2091 were used as growth controls for the nutrient agar and potato dextrose agar, respectively.

Mutagenic Studies:

Sample Preparation: Dried *K. senegalensis* stem bark was pulverized and extracted by decoction. Fifty grams (50.0 g) of pulverized material was boiled in 500 mL of distilled water for 20 min. The decoction was centrifuged at 6000 RPM for 10 min. The supernatant was collected and concentrated under vacuum at 40 ºC and lyophilized to obtain dried crude extracts. The extract was stored at -20 ºC for use. The samples were prepared and diluted with sterile water on the day of the assay to a concentration of 10 µg/mL and sterile filtered using a 0.22 µM membrane filter.

Induction of Mutation: The Muta-ChromoPlate™ kit (manufactured by Environmental Bio-Detection Products Inc, Ontario, Canada), which works on the principle of the Ames test [38] was employed to determine the mutagenicity potential of the extracts. The experiment was carried out in accordance with protocol provided by the manufacturer using *Salmonella typhimurium* TA98. Similar methods are reported in published literature [36].

The level of mutagenicity was detected as an increase in the number of histidine revertants with reference to scores provided in the kit. The statistical table provided in the kit was used to compare the natural background rate of reverse mutation to the rate of reverse mutation within a sample assay. The number of positive wells scored in the 96 well plates determined the significance of mutation in the fluctuation test [39].

The statistical table provided in the kit was used to compare the natural background rate of reverse mutation to the rate of reverse mutation within a sample assay. Based on these samples, p values of 0.001 were classified as highly mutagenic, p values of 0.01 moderately mutagenic, and p values of 0.05 weakly mutagenic. Mutation in the TA 98 is indicative of a frameshift mutation (FSM).

RESULTS AND DISCUSSION:

Macroscopic and Microscopic Characteristics: A summary of the organoleptic and macroscopic characteristics of both the leaves **Table 1** and the stem bark of *K. senegalensis* **Table 2** are provided below. **Fig. 1** also displays the pinnately compound leaves and unique red inner colored bark and grey outer-bark of this plant. The estimated constants of the leaves, such as vein islet number, veinlet termination number, and paracytic stomata are characteristics of the leaves which can be used in the rapid authentication of the plant. This can be used in identifying *K. senegalensis* before the bark samples are harvested for processing. The characteristic features of green-grey outer bark and reddish-brown inner bark, characteristic odor, bitter taste, and short-splintery fracture, which are simple means of further confirming the identity and quality of the stem bark of *K. senegalensis*.

TABLE 1: MACROSCOPIC CHARACTERISTICS OF *K. SENEGALENSIS* LEAVES

Morphology	Description
Type	Pinnately compound
Shape	Oblong to obovate
Colour	Green
Arrangement	Alternate
Venation	Pinnately reticulate
Base	Decurrent
Apex	Mucronate
Margin	Entire
Surface	Glabrous
Texture	Smooth and papery

TABLE 2: ORGANOLEPTIC CHARACTERISTICS OF THE STEM BARK OF *K. SENEGALENSIS*

Morphology	Description
Condition	Dried
Outer bark color	Dark-grey
Inner bark color	Reddish-brown
Odor	Slight
Taste	Astringent
Texture	Scaly
Fracture	Short-splintery

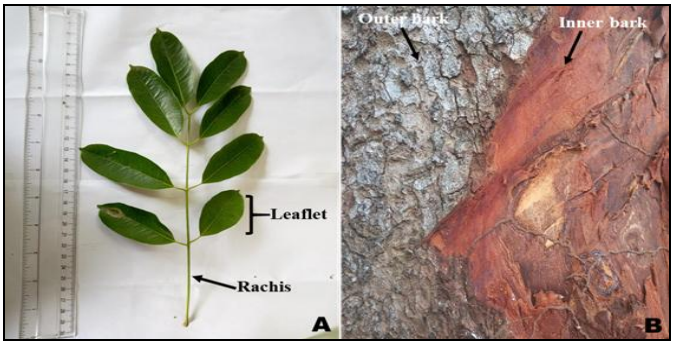


FIG. 1: LEAVES AND STEM BARK OF *K. SENEGALENSIS*

Microscopic analysis of the leaves of *K. senegalensis* showed an average vein islet number of 24.9, vein islet termination number of 26, stomata number of 5.3, and number of epidermal cells of 173.3 per mm^2 as indicated in **Table 3**. **Fig. 2** of the leaves of *K. senegalensis* displays the epidermal cells and cystoliths, veinlet terminations and vein islets, calcium oxalate crystals, stomata cells, palisade cells, and branched dendritic trichomes.

TABLE 3: LEAF CONSTANTS

Parameter	*K. senegalensis*
Stomatal Number	5.3
Epidermal Cell Number	173.3
Vein islet Number	24.9
Veinlet termination number	26.0
The stomatal index [stomata per square mm of epidermis]	2.97

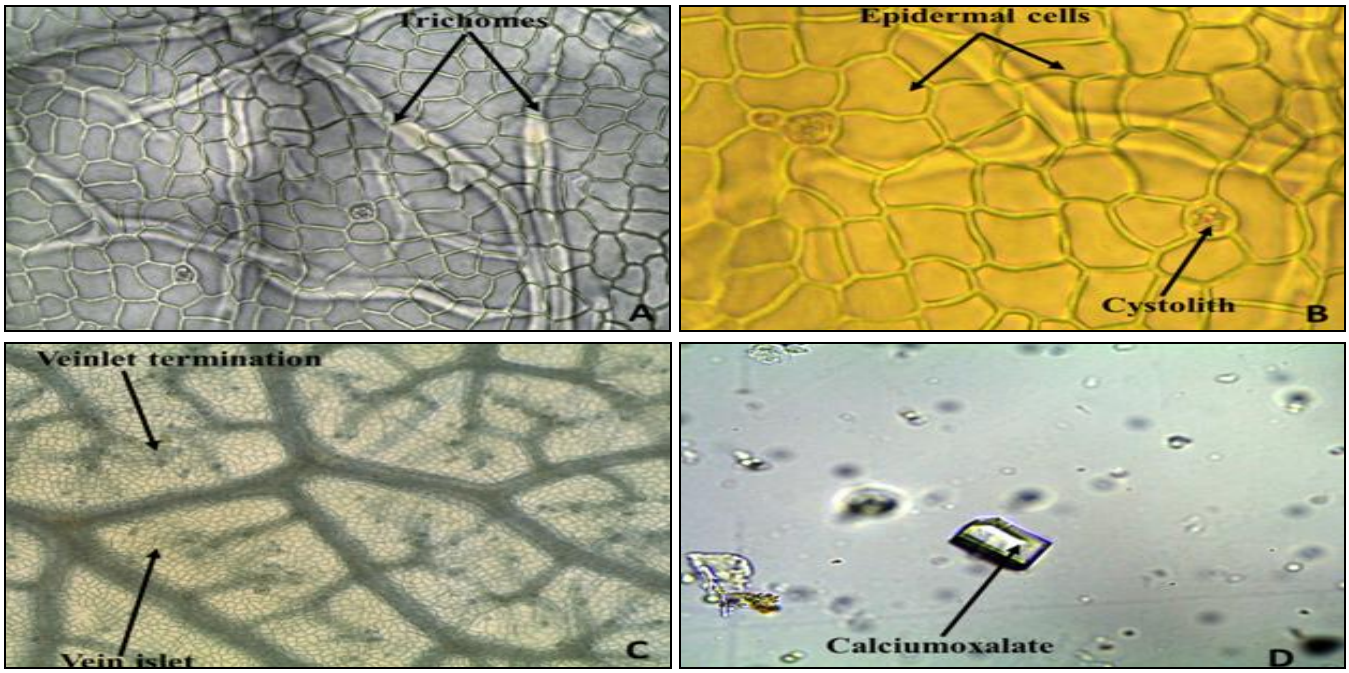


FIG. 2: MICROSCOPIC CHARACTERISTICS OF LEAF EPIDERMIS AND POWDERED LEAF OF *K. SENEGALENSIS*. A. HIGHLY BRANCHED TRICHOMES; B. WAVY EPIDERMAL CELLS AND CYSTOLITHS. C. VEINLET TERMINATIONS AND VEIN ISLETS. D. PRISMATIC CALCIUM OXALATE CRYSTAL

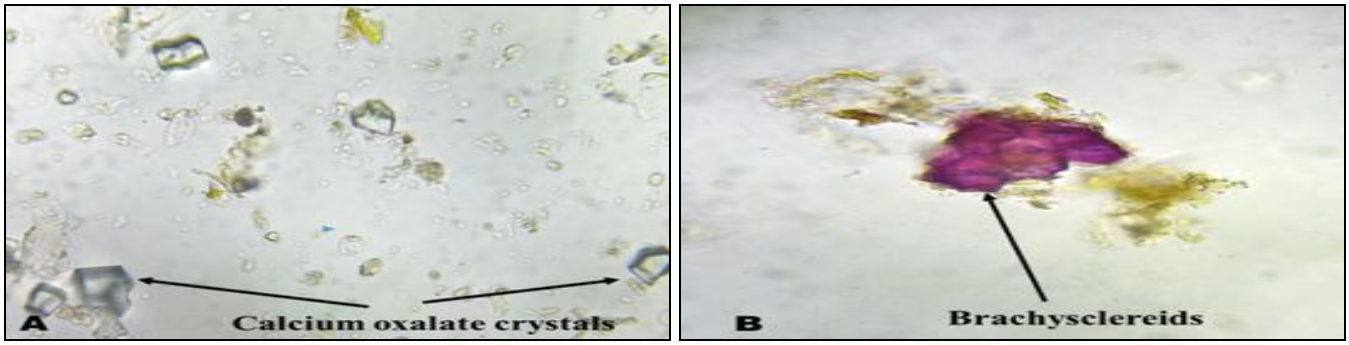


FIG. 3: MICROSCOPIC FEATURES OF THE POWDERED STEMBARK OF *K. SENEGALENSIS*. A. SHOWS PRISMATIC CALCIUM OXALATE CRYSTALS; B. SHOWS A CLUSTER OF BRACHYSCLEREIDS

Powdered Microscopy of Stem Bark: Microscopic investigation of the powdered stem bark revealed the presence of prismatic calcium oxalate crystals, which were in abundance, as well as brachy-sclereids, which were occurring in clusters (refer to **Fig. 3**).

Physico-chemical and Phytochemical analysis: Physico-chemical constants that were determined for the bark of *K. senegalensis* included the ash values and foaming index of 142.8. Details are provided in **Table 4**. The air-dried stem bark had an average moisture content of 5.47% w/w, indicating that a significantly higher moisture content than this value could be indicative of a poor quality material because this could hasten moisture and microbial decomposition which detrimentally affect the quality. *K. senegalensis* had no swelling index, possibly indicating the absence of mucilage, pectin or hemicellulose which have swelling properties [40]. Its foaming index was 142, thus indicating the possible presence of saponins which caused persistent foam when the aqueous decoction is shaken [40].

TABLE 4: ASH VALUE PARAMETERS

Parameters	*K. senegalensis* (% w/w)
Total Ash	6.53
Acid insoluble ash	2.13
Water-soluble ash	6.6
Moisture content	5.47
Foreign organic matter	Nil
Foaming index	142.85
Swelling index	-

K. senegalensis yielded extractive values of 11.8, 15.1, and 1.9% w/w for water, ethanol, and petroleum ether, respectively. *K. senegalensis* had a higher proportion of middle polar constituents (11.8% w/w in ethanol) significantly as compared to polar constituents in water (11% w/w) and non-polar constituents in petroleum ether (15% w/w).

The extracts tested positive for alkaloids, tannins, glycosides, saponins, flavonoids, and steroids.

TABLE 5: EXTRACTIVE VALUES

Parameters	*K. senegalensis* (% w/w)
Petroleum ether	1.98
50% ethanol	15.16
Water	11.80

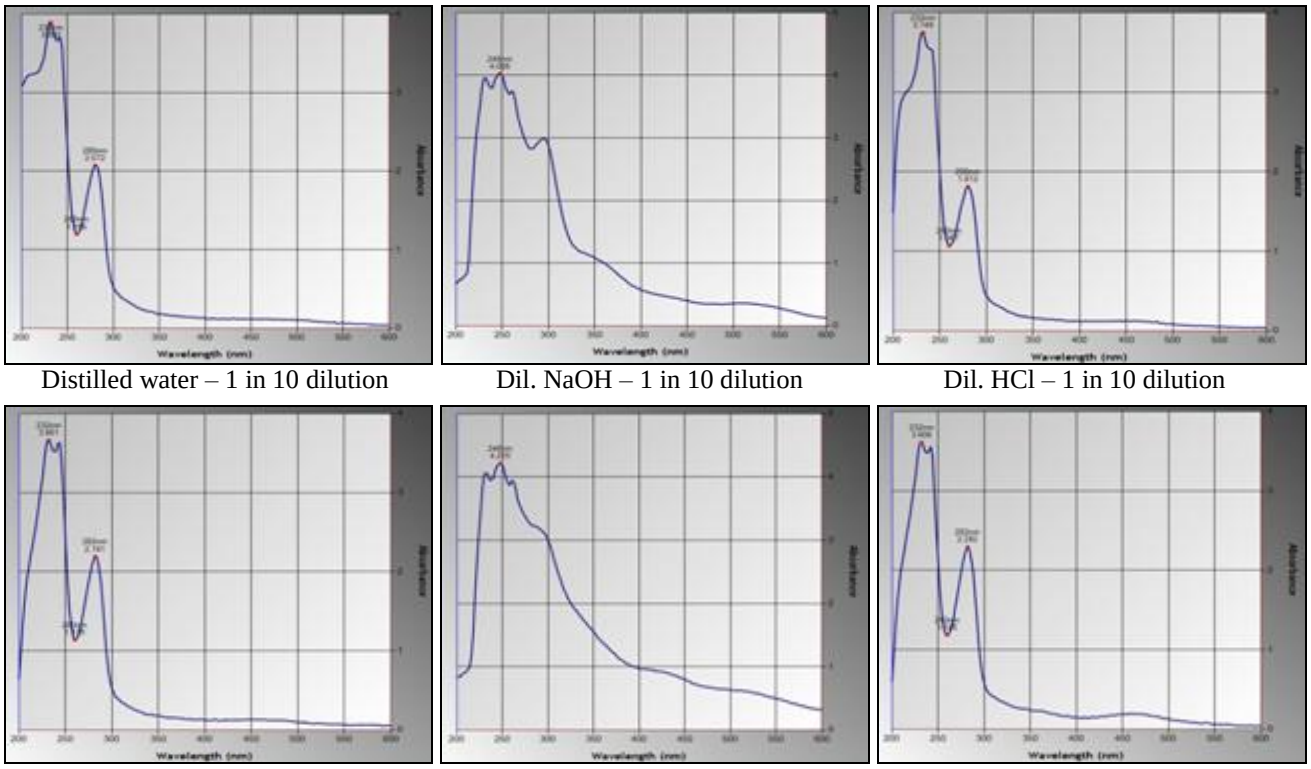
Fluorescence Analysis: Analysis for characteristic fluorescence of the plant material at the short, and long ultra-violet wavelengths of λ 254 nm and λ 365 nm showed colors as provided in Table 6. Fluorescence is the ability of a substance to emit light that has been absorbed. The light emitted depends on the wavelength of emission and the type of compound involved. This can, therefore, be used to qualitatively assess the components of herbal medicines. For crude herbal drugs, this can be seen in the form of bright colors under short or long wavelengths of ultra-violet light. The characteristic fluorescence of *K. senegalensis* at these wavelengths could be used as a further confirmation of the identity, purity, and quality of the plant sample. For example, in methanol the plant sample is wine under daylight, deep blue under at 254, and ash at 365 nm.

TABLE 6: FLUORESCENT STUDIES OF POWDERED STEM BARK OF *K. SENEGALENSIS* IN VARIOUS SOLVENTS

	Daylight	254 nm	365 nm
Powdered Sample only	Reddish-brown	Dark brown	Dark brown
Distilled water	Light brown	Deep blue	Pale blue
1N HCl	Orange	Deep blue	Pale blue
1N NaOH	Black	Deep blue	Pale blue
50 % H_2SO_4	Dark Brown	Deep blue	Pale blue
Methanol	Wine	Deep blue	Ash
Glacial acetic acid	Orange	Purple	Light purple
Nitric acid	Wine	Deep blue	Light purple
Chloroform	Dark brown	Deep blue	Light purple
50 % $FeCl_3$	Deep green	Deep blue	Pale blue
95 % Ethanol	Cherry red	Deep blue	Pale blue

UV Spectra Analysis: The UV spectra of the aqueous extract of the stem bark of *K. senegalensis* dissolved in various solvents of different pHs, showed spectra shifts under acidified and basic conditions and changes in absorbance levels (refer to **Fig. 4**). This indicates that component (s) of the crude extract of *K. senegalensis* absorb ultraviolet/ visible light of a wavelength of 233 and 280 nm. There are slight shifts in λ_{max} for the basified extract in dilute NaOH and in HCl but none in Methanol. Similar patterns are seen in the ethanol extracts **Fig. 5**. The wavelength at which absorbance is the highest is called the lambda max (λ_{max}), and it is characteristic of particular groups of constituents of the extract. This pattern over a wavelength range can be used for substantiating the identification of crude medicinal plants since this value can be fairly constant and only varying with a change in composition of the plant material. Hence the UV absorption spectrum over the aqueous

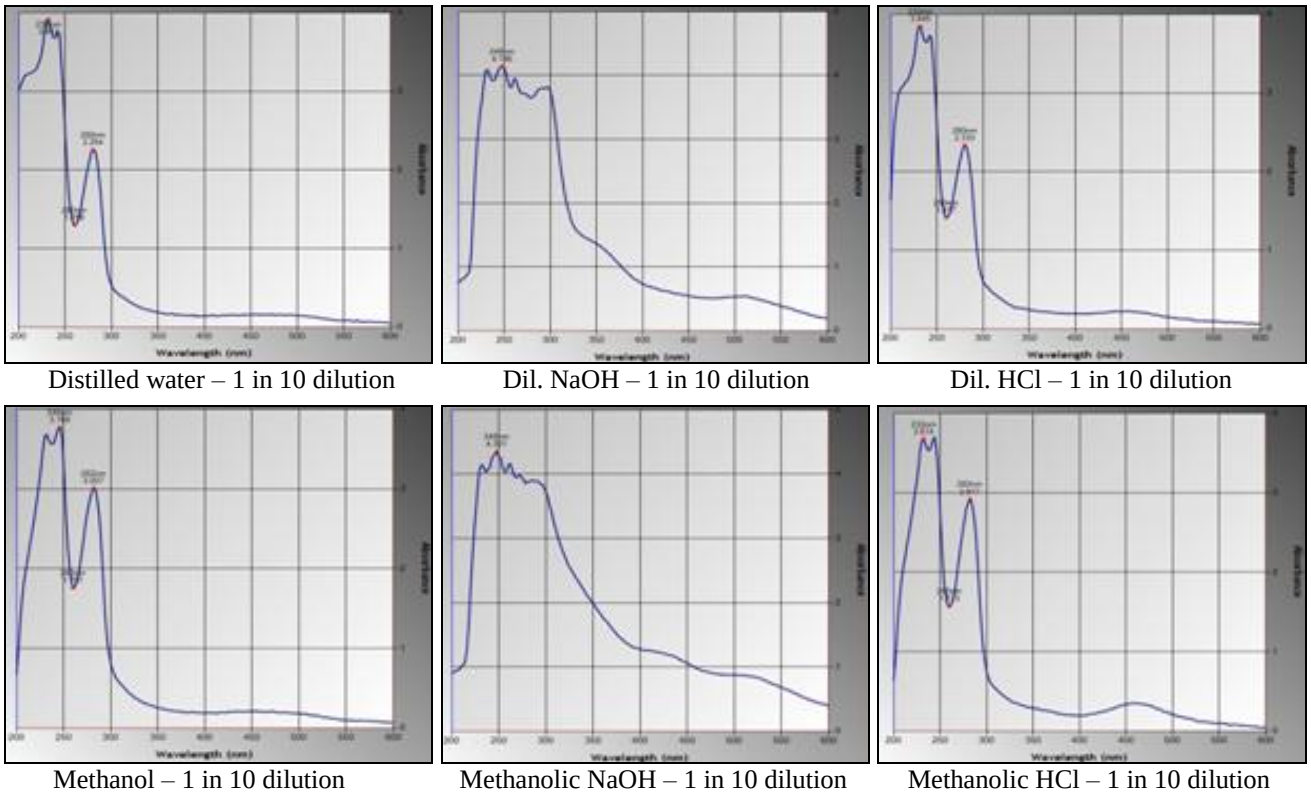
extract from 200 to 600 nm and λ_{max} of 3.8 at 233 nm and 2.7 at 280 nm can be used to authenticate samples of *K. senegalensis*. Authentication can be further established by observing the slight shifts in absorbance in dilute acid and in the dilute base, as seen in **Fig. 4**. Similar patterns can also be observed with the ethanol extract.



Distilled water – 1 in 10 dilution | Dil. NaOH – 1 in 10 dilution | Dil. HCl – 1 in 10 dilution

Methanol – 1 in 10 dilution | Methanolic NaOH – 1 in 10 dilution | Methanolic HCl – 1 in 10 dilution

FIG. 4: UV SPECTRA OF AQUEOUS EXTRACT OF *K. SENEGALENSIS* IN VARIOUS SOLVENTS



Distilled water – 1 in 10 dilution | Dil. NaOH – 1 in 10 dilution | Dil. HCl – 1 in 10 dilution

Methanol – 1 in 10 dilution | Methanolic NaOH – 1 in 10 dilution | Methanolic HCl – 1 in 10 dilution

FIG. 5: UV SPECTRA OF ETHANOL EXTRACT OF *K. SENEGALENSIS* IN VARIOUS SOLVENTS

Heavy Metal Content: When the powdered stem bark of *K. senegalensis* was analyzed, it showed traces of heavy metals such as Cadmium (Cd), Lead (Pb), *etc*. (refer to **Table 7**), but the levels were within recommended limits [40].

TABLE 7: HEAVY METAL CONTENT

Metal	*K. senegalensis* (µg/kg)
Cadmium (Cd)	0.0029
Lead (Pb)	0.0085
Iron (Fe)	0.0466
Zinc (Zn)	0.0009
Arsenic (As)	-

Microbiological and Mutagenic Analysis: Analysis for aerobic bacteria and fungi put an estimated count of aerobic bacterial and fungal cells at both 10^4 cells per gram of dried plant material. It is recommended that, for plants materials to which boiling water would be added, such as *K. senegalensis*, aerobic bacteria and fungi should be less than $\leq 10^5$ and $\leq 10^4$ [41]. Hence the microbial counts of this plant material were within range. Precaution would need to be exercised when processing such materials so that the microbial counts do not exceed the recommended value because this could cause degradation of the product and also reduce its efficacy, safety, and quality. *K. senegalensis* exhibited weak mutagenicity in the Ames test.

CONCLUSION: The botanical, chemical, biological, and physical parameters determined could be pivotal in establishing the quality of crude plant materials of *K. senegalensis* by such available methods before they are used as medicines.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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