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Pharmacognostic standardization and qualitative analysis of *Gymnosporia senegalensis*

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Abstract: This study aims to evaluate the pharmacognostic parameters of different parts of *Gymnosporia senegalensis* (Lam.) Loes. These parameters play a vital role in the traditional medicine system for administering the drug and their therapeutic effects on various ailments. Standardization parameters include sequential extract preparation, physicochemical studies (ash value, moisture content, pH, fluorescence analysis), and phytochemical screening of the three parts of *G. senegalensis*. Various macro and microelements (in ppm) were estimated by using atomic absorption spectroscopy (AAS). Total ash content (3.04±0.02), water-soluble ash (2.63±0.01), and insoluble acid ash (0.72±0.01) were found to be the highest in the leaf. Foreign organic matter was observed higher in stem (0.07±0.04) followed by bark and leaf. The moisture content was found lowest in bark (2.02±0.01), preventing microbial growth. The pH of the crude drug of plant parts is weakly acidic, ranging from pH 5.33 to 5.96 at 1 % and 4.93 to 6.03 at 10 %. Fluorescence results were also helpful in detecting the substituents and adulterants and assessing the crude drugs qualitatively. The present study revealed the presence of various primary and secondary metabolites (terpenoids, flavonoids, tannins, and saponin) in high and moderate amounts in the extracts of different parts of the plant. The stem and bark also showed a reasonable presence of macro and microelements (As, Cr, Cd, Pb, Zn, Mn, and Cu). The complete analysis provides valuable information for the quality assurance of *G. senegalensis* as a crude drug for preparing formulations of herbal medications.

Keywords: atomic absorption spectroscopy; heavy metals; phytochemical screening; quality control; secondary metabolites

1. Introduction

The quality control of therapeutic drugs is a fundamental criterion that must be considered. At the same time, coping with plant products that are supposed to be placed on the market as an element for drug composition in basic pharmacological investigations. Therefore, efforts should be taken to achieve and preserve the superior quality of these medicinal herbs. The importance of medicinal plants is well-known, and human beings have used them for various purposes [1]. In recent years, medicinal plants have obtained numerous natural bioactive compounds with curative values and treat many diseases as alternative medicines [2-4]. Traditional herbal remedies have constantly led scientists to explore new drugs to facilitate the safety of humans and animals. However, lack of standardization is a significant drawback in the preparation and utilization of herbal medicines.

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The problem relating to the standardization of natural drugs ascends from the multifaceted composition of medicines used in the whole plant, plant parts, or extract obtained (in both its entire and powder form). Some plants contain critical therapeutic properties, but the benefits remain confined primarily because of a lack of awareness and improper scientific standardization. Therefore, plant material standardization is a prior requirement [5].

It assures quality, acceptability, safety, purity, efficacy, and the identity of the herbal formulations by reducing batch-to-batch variation to make them valuable to consume. Pharmacognostical studies were carried out based on a detailed botanical evaluation of the plants, which included botanical parameters with physicochemical and pharmacognostic analysis [6], providing an opportunity to identify and evaluate authentic herbs with an excessive amount of bioactive components. Various pharmacopeia has featured the plant components in the form of monographs to classify the physicochemical characteristics. Thus, the advanced approach to identifying and quantifying the active ingredients can help in the appropriate standardization of the herbs and their formulations in the plant materials [7, 8].

Gymnosporia senegalensis (Lam.) Loes (family - Celastraceae) is a small tree or tall shrub usually distributed

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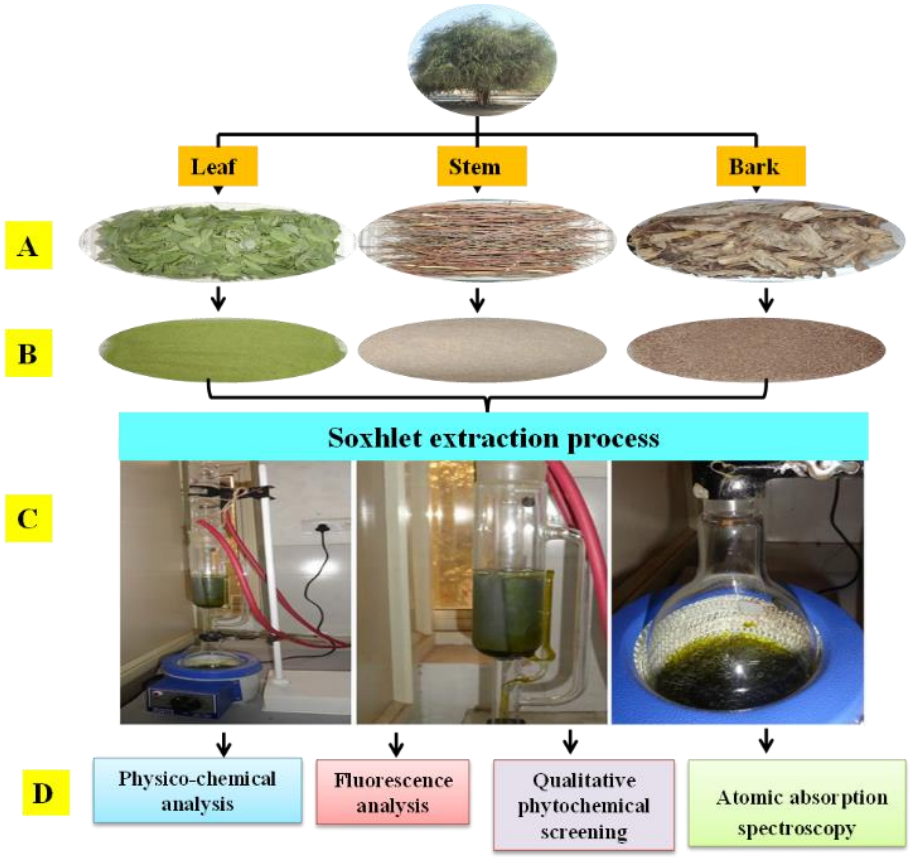

Figure 1. Preparation of extracts by Soxhlet hot continuous method; A) fresh leaf, stem, and bark of *Gymnosporia senegalensis*; B) powdered form of all parts of the plant; C) sequential extract preparation of all the parts with the help of Soxhlet apparatus; D) fresh powder and extract prepared and utilized for the parameters.

in Afghanistan, Africa, Arabia, and India [9]. The root, stem, and bark of *G. senegalensis* have been used to treat several ailments like diarrhea, chest pains, rheumatism, chronic illness, wounds, dyspepsia, eye infection, dysmenorrhoea, and malaria [10-12]. Scientific reports have also confirmed its anti-inflammatory and antimicrobial activity [13].

Lack of sufficient documentation and the implementation of reliable quality control methods remain critical hindrances in using herbal drugs, laying the groundwork for misinterpretation, the unintended substitution of similar species, and deliberate tampering with authentic herbs with poor quality to fulfill the growing consumer demands [8]. Despite the broad utilization of *G. senegalensis*, standardization parameters presented in the literature are not quite informative for its proper documentation and identification. Therefore, we have conducted this study to establish some standard pharmacognostic parameters of *G. senegalensis* to prepare its herbal drugs.

2. Experimental

2.1 Procurement and validation

The different parts (leaf, stem, and bark) of *G. senegalensis* were collected from nearby areas of Banasthali, Tehsil Newai, Rajasthan, India, in January 2019. Patanjali Herbal Research Department, Haridwar, India, identified and authenticated the plant parts. For further reference, an herbarium specimen having a serial number PRFH/005 was deposited at the Patanjali Research Foundation herbarium. The scheme of work undertaken is shown in **figure 1**.

2.2 Preparation of extracts

Fresh leaf, stem, and bark of *G. senegalensis* were washed, shade dried at room temperature for nearly one month, and milled into homogenous powder using an electric grinder (Panasonic MKGW200). The powder was passed through the 40-mesh sieve and stored at room temperature for further examination. Fifty grams of dried powder of all the plant parts were used to prepare sequential extracts using a hot continuous soxhlation extraction method with different solvents in their ascending order of polarity ranging from non-polar to polar. The Soxhlet mixture was filtered, evaporated, and stored at 4 °C in an airtight container for further use [14-16].

2.3 Physicochemical analysis

The physicochemical parameters such as the ash content (total ash, water-soluble ash, and acid-insoluble ash), foreign organic matter, loss of dryness (LOD), and pH (at 1 % and 10 %) are the deciding factors in evaluating impurity, purity, and quality of the crude drugs. All the analyses were

performed according to the WHO guidelines on quality control methods for medicinal plant materials [17].

2.4 Fluorescence analysis

A minute amount of powdered crude drug samples was placed in the test tubes with various solvents and chemical reagents (acidic and basic). Different fluorescence color radiations emitted by a range of solutions were noted under visible, short, and long UV wavelength regions [18].

2.5 Qualitative phytochemical screening

Preliminary phytochemical screenings of different solvent extracts of *G. senegalensis* for identifying various active ingredients were carried out using standard conventional procedures [14].

2.6 Atomic absorption spectroscopy (AAS)

The digestion method was used for the analysis of elements including arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn), manganese (Mn), and copper (Cu) in plant parts (leaf, stem, and bark). Two grams of each sample were acidified with 15 ml of 65 % (v/v) conc. nitric acid and oxidized with 5 ml of 30 % (v/v) hydrogen peroxide. The solutions were heated for 3 h at 250 °C in a muffle furnace until the digest was clear. The solution was cooled down and filtered through Whatman No. 42 filter paper, transferred quantitatively into a flask, and made up to the mark by adding distilled water. These samples were analyzed using a model iCE 3300 GF high-resolution atomic absorption spectrophotometer. The instrument was calibrated with the help of standard solutions of As, Cr, Cd, Pb, Zn, Mn, and Cu to analyze these elements at specific wavelengths. Air-acetylene flame as gas pressure and hollow cathode as lamp source at 50 mm burner with different gas flow rates (L/min) were used as optical analytical conditions [19].

2.7 Statistical analysis

All the experiments were carried out in triplicates, and the values were represented as mean ± standard deviation (SD).

3. Results and discussion

In this study, we have standardized various phytochemical and physicochemical properties of the leaf, stem, and bark of *G. senegalensis*. The results of standardization parameters are:

3.1 Extract preparations of *G. senegalensis*

The percentage yields (% w/w) of the extracts of *G. senegalensis* (leaf, stem, and bark) were analyzed using different solvents. It indicated the presence of various bioactive compounds in all extracts from the experimental plant. The results revealed that in the case of leaf and stem, the polar solvent methanol has a high extractive yield (17.86±0.03 and 9.84±0.01), whereas, in the case of bark,

Table 1. Preliminary phyto-profile of various parts (leaf, stem and bark) of *G. senegalensis* in different solvents.

Solvents	CE (After Soxhlation extraction)	CE (1 mg/ml)	C	Nature	pH	% Yield
Leaf						
Petroleum ether	Dark green	Yellowish	Dry	Semi-solid	4	11.39±0.13
Benzene	Dark green	Olive green	Oily	Solid	6	2.89±0.01
Chloroform	Fluorescent green	Pale green	Dry	Solid	4	8.19±0.03
Ethyl acetate	Olive green	Pale off green	Sticky	Solid	4	1.70±0.02
Methanol	Dark brown	Light green	Sticky	Solid	6	17.86±0.03
Distilled Water	Dark brown	Dark brown	Dry	Semi-solid	4	3.19±0.01
Stem						
Petroleum ether	Olive green	Pale green	Oily	Semi-solid	5	1.52±0.02
Benzene	Green	Green	Dry	Solid	6	0.58±0.01
Chloroform	Green	Olive green	Dry	Solid	5	3.08±0.03
Ethyl acetate	Pale green	Light green	Sticky	Solid	5	1.15±0.02
Methanol	Reddish brown	Light brown	Sticky	Solid	6	9.84±0.01
Distilled Water	Dark brown	Dark green	Dry	Semi-solid	4	6.65±0.03
Bark						
Petroleum ether	Yellowish orange	Orangish	Oily	Semi-solid	5	6.10±0.20
Benzene	Olive green	Green	Dry	Solid	6	1.30±0.05
Chloroform	Olive green	Olive green	Dry	Solid	4	0.42±0.01
Ethyl acetate	Pale green	Green	Sticky	Semi-solid	6	0.55±0.00
Methanol	Reddish brown	Yellowish	Sticky	Solid	6	2.29±0.01
Distilled Water	Dark brown	Light brown	Dry	Semi-solid	6	1.44±0.01

Means of three replicates ± SD; SD: Standard deviation of the mean, CE: Colors of extracts, C: Consistency

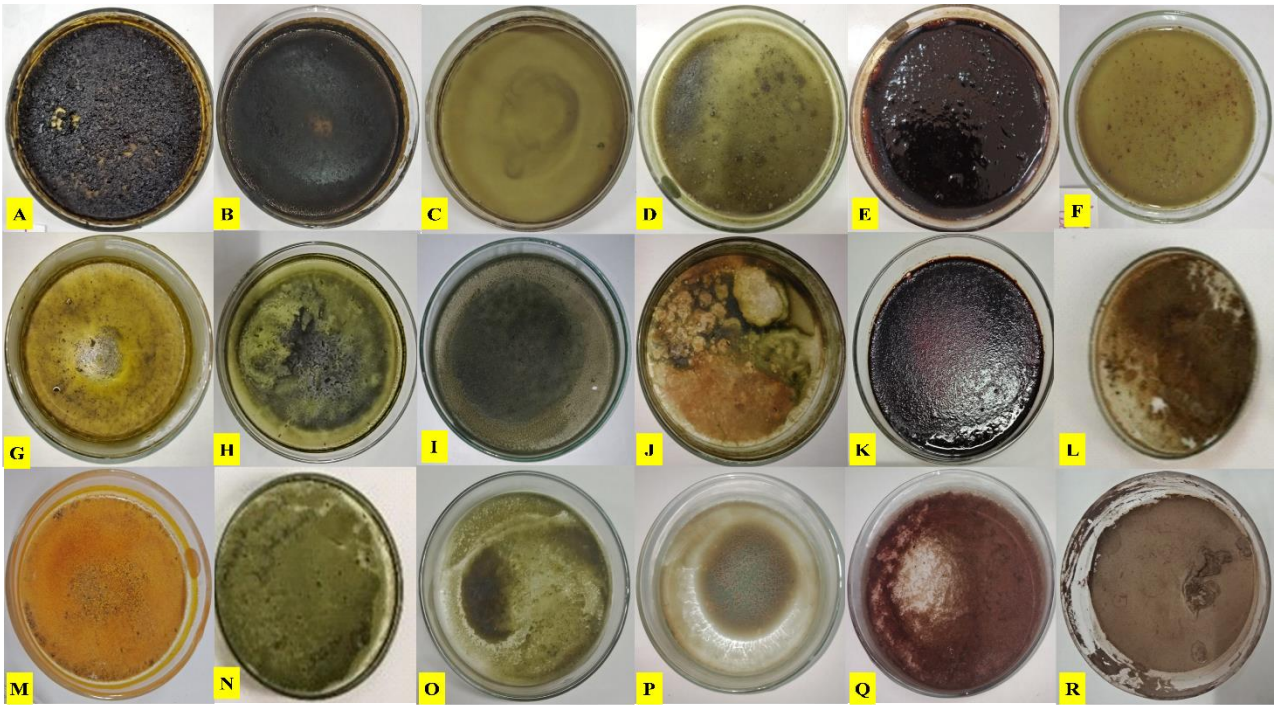

Figure 2. Sequential extracts of leaf (A: Petroleum ether, B: Benzene, C: Chloroform, D: Ethyl acetate, E Methanol, F: Aqueous); stem (G: Petroleum ether, H: Benzene, I: Chloroform, J: Ethyl acetate, K: Methanol, L: Aqueous) and bark (M: Petroleum ether, N: Benzene, O: Chloroform, P: Ethyl acetate, Q: Methanol, R: Aqueous) of *G. senegalensis*.

the non-polar solvent petroleum ether has a high extractive yield (6.10±0.20). The extractive values help evaluate the existence and nature of bioactive constituents present in solvent extracts, as well as in the assessment of the solubility of specific bioactive components in different solvents. These results also indicated that the amount of metabolites varied from high to low in the leaf, stem, and bark extracts, respectively. The pH of leaf, stem, and bark extracts (1 mg/ml) was weakly acidic (pH~5-6). The color, consistency, nature, and pH of extracts from different parts were analyzed and listed in **table 1** and shown in **figure 2**.

3.2 Physicochemical analysis

Physicochemical parameters help ensure crude drug purity, quality, efficacy, and safety. The ash value of *G. senegalensis* was determined by three different procedures, i.e., total ash, water-soluble ash, and acid-insoluble ash. Ash constituents are the inorganic residues (such as silicates, carbonate, oxalate, and silica) obtained after the complete combustion of a crude drug. The total ash value (TAV) contains physiological and non-physiological ash. Physiological ash presents naturally in plant tissues and synthesizes itself by several biochemical processes. The non-physiological ash is a kind of contamination that accumulates in the plant parts from the atmosphere, mainly by absorbing soil [20].

In the present study, the TAV was noted to be the highest in the leaf (3.04±0.02), with respect to the stem (2.14±0.02) and the bark (1.42±0.01). A high percentage of TAV revealed the inorganic constituents in the crude sample of *G. senegalensis*. Further, water-soluble ash (WSA) is a significant marker for detecting water-soluble salts and other materials in the crude sample. In the case of *G. senegalensis*, the highest WSA was observed in the leaf (2.63±0.01), w.r.t. bark (2.01±0.01), and the stem (1.74±0.01). Moreover, acid insoluble ash (AIA) is a part of total ash that remains insoluble in dil. HCL and known as non-physiological ash especially having sand and soil contents. In the case of our samples, the highest AIA was observed in the leaf (0.72±0.01), w.r.t. the stem (0.65±0.03), and the bark (0.15±0.01). Its lowest value indicates the presence of a negligible amount of siliceous matter.

Foreign organic matter states the presence of any matter or adulteration that does not form the part of a crude drug and can impart as impurities in the crude extract. It was observed higher in stem (0.07±0.04) and bark (0.08±0.03) as compared to leaf (0.02±0.01). It helps in determining the adulteration present in the crude drug. Moisture content is also essential in crude drugs, directly correlated with its reliability and stability. The higher moisture content must be avoided in the formulations of drugs to prevent their deterioration. The lower level of moisture can prevent microbial growth [21]. The moisture content of *G. senegalensis* was determined by the loss on drying method (LOD) at 105 °C and found to be 3.12±0.01 for leaf and 2.84±0.02 for the stem, whereas 2.02±0.01 for bark. The pH of the crude drug of plant parts is weakly acidic, ranging from 5.33 to 5.96 at 1 % and 4.93 to 6.03 at 10 %, respectively. Thus, it may not trigger any gastrointestinal inflammation [22]. The physical state, color, and taste of the dry ash collected after careful incineration and their proximate analysis of the leaf, stem, and bark of *G. senegalensis* are given in **table 2** and graphically represented in **figure 3**.

Table 2. Physicochemical analysis of leaf, stem and bark of *G. senegalensis*.

Characterization		Leaf	Stem	Bark
Physical state of ash		Fine powder	Fine powder	Fine powder
Color of ash		Grey	Grey	Grey
Taste of ash		Alkaline	Alkaline	Alkaline
Total ash value (%WW)		3.04±0.02	2.14±0.02	1.42±0.01
Water soluble ash value (%WW)		2.63±0.01	1.74±0.01	2.01±0.01
Acid insoluble ash value (%WW)		0.72±0.01	0.65±0.03	0.15±0.01
Foreign organic matter (%DW)		0.02±0.01	0.07±0.04	0.08±0.03
Loss on drying (%DW)		3.12±0.01	2.84±0.02	2.02±0.01
pH	1%	5.63±0.11	5.96±0.05	5.33±0.57
	10%	6.03±0.11	4.93±0.11	5.66±0.35

Mean value ± Standard deviation of triplicate results

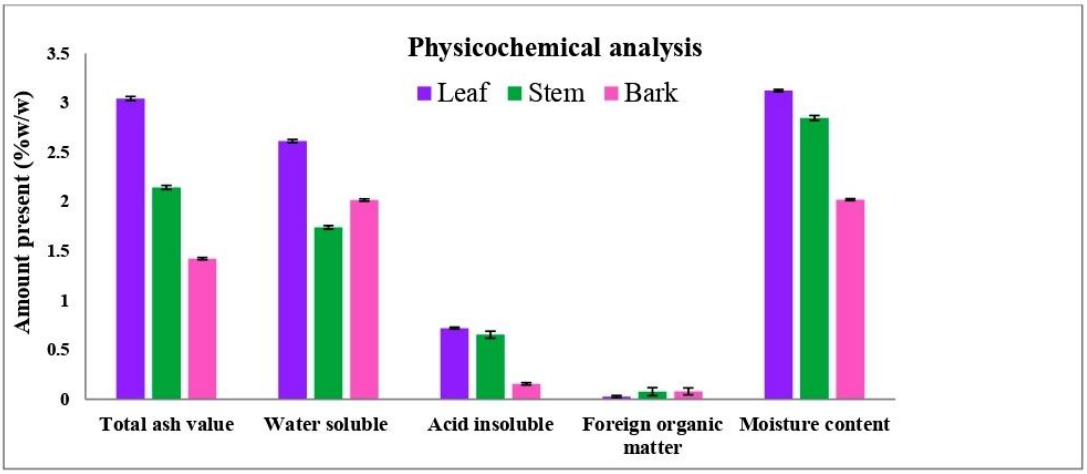

Figure 3. Evaluation of various physicochemical analyses of leaf, stem, and bark of *G. senegalensis*.

3.3 Fluorescence characterization

Fluorescence is the observable fact exhibited by a variety of chemical compounds present in the plant sample. Ultraviolet light produces fluorescence in many natural drugs or after treating them with various reagents. If the sample itself is not fluorescent, it may be changed into multiple fluorescent derivatives after applying different reagents. The results of fluorescence analysis of other parts (leaf, stem, and bark) of *G. senegalensis*, after treating them with various acidic and basic chemical reagents and solvents under visible, short (254 nm), and long (365 nm) UV light, are given in **table 3**. This study helps qualitatively detect the substituents and contaminants and assess the crude drugs [23].

3.4 Phytochemical screening

The results of preliminary phytochemical screening of various solvent extracts of leaf, stem, and bark of *G. senegalensis* revealed that the plant possesses primary metabolites such as proteins, amino acids, carbohydrates, and lipids in trace amounts, whereas secondary metabolites such as alkaloids, flavonoids, terpenoids, and glycosides, etc., in appreciable quantity. These metabolites were obtained in petroleum ether, chloroform, ethyl acetate, and methanol extract from *G. senegalensis*, indicating that these solvents effectively isolated the specific bioactive compounds.

3.4.1 Primary metabolites

Proteins and amino acids were observed in higher amounts in chloroform extract of leaf, in an equal ratio of ethyl acetate and methanol extract of the stem, and the benzene extract of bark. Carbohydrates, the third primary biopolymers, were in high amounts in chloroform and methanol extract in all parts of *G. senegalensis*, whereas in moderate quantities in other solvent extracts. Lipids such as oils, fats, and essential oils were absent in the aqueous extract of all parts of *G. senegalensis*, whereas these were present in other extracts. Lipids possess many biological activities such as antifungal, antibacterial, antioxidant, antiviral, antimicrobial, etc. [24, 25]. These are also safe for animal and human consumption [26].

Table 3. Fluorescence characteristics of different plant parts (leaf, stem and bark) of *G. senegalensis.*

Reagent's used	Leaf			Stem			Bark		
	Short UV (254 nm)	Long UV (365 nm)	Ordinary light	Short UV (254 nm)	Long UV (365 nm)	Ordinary light	Short UV (254 nm)	Long UV (365 nm)	Ordinary light
Powder as such (P)	Fluorescent green	Dark green	Green	Creamish	Brown	Creamish	Dark green	Dark brown	Dark brown
P + Picric acid	Fluorescent green	Black	Light green	Pale green	Dark brown	Yellowish green	Green	Black	Light green
P + Glacial acetic acid	Dark green	Purple	Light green	Green	Pale brown	Creamish	Dark green	Black	Dark brown
P + HCl	Dark green	Black	Dark green	Dark green	Black	Black	Black	Black	Black
P + H_2SO_4	Fluorescent green	Black	Dark green	Black	Black	Black	Black	Black	Black
P + HNO_3	Dark green	Black	Brown	Dark green	Dark orange	Dark brown	Light green	Black	Yellowish
P + $FeCl_3$ (5%)	Dark green	Dark green	Light green	Green	Dark green	Pale green	Light green	Black	Light green
P + Iodine solution (5%)	Dark green	Dark green	Fluorescent green	Green	Dark brown	Brown	Dark green	Dark green	Dark brown
P + Ammonia solution	Dark green	Black	Fluorescent green	Dark green	Black	Brown	Dark green	Black	Brown
P + NaOH	Dark green	Black	Light green	Dark green	Black	Brown	Dark green	Black	Brown
P + Potassium dichromate	Dark green	Black	Brown	Dark green	Dark purple	Orangish	Green	Black	Orangish
P + Toluene	Dark green	Dark green	Light green	Pale brown	Dark brown	Brown	Black	Black	Dark brown
P + Petroleum ether	Dark green	Dark green	Fluorescent green	Pale green	Light brown	Brown	Dark green	Black	Dark brown
P + Benzene	Fluorescent green	Dark green	Pale green	Green	Dark brown	Brown	Black	Black	Light brown
P + Chloroform	Dark green	Dark green	Fluorescent green	Dark green	Dark brown	Brown	Dark green	Dark black	Dark brown
P + Ethyl acetate	Light green	Dark green	Fluorescent green	Pale green	Brown	Brown	Black	Black	Dark brown
P + Distilled water	Dark green	Dark green	Light green	Green	Brown	Light brown	Dark brown	Dark green	Dark brown
P + Acetone	Green	Orangish	Pale green	Light green	Light brown	Light brown	Dark green	Black	Dark brown
P + NH_3 + HNO_3	Pale green	Dark green	Yellowish	Green	Dark brown	Orange	Fluorescent green	Black	Light brown
P + Ethanol	Dark green	Dark green	Light green	Pale green	Dark brown	Light brown	Black	Dark green	Dark brown
P + Methanol	Dark green	Black	Green	Green	Dark brown	Brown	Dark brown	Black	Dark brown
P + Dragendorf reagent	Light green	Dark green	Light brown	Dark black	Pale green	Pale green	Dark green	Black	Brown
P + Phenol reagent	Black	Black	Dark blue	Black	Black	Dark blue	Dark green	Black	Green
P + Folin and Ciocalteu's reagent	Green	Dark green	Black	Black	Black	Dark blue	Black	Black	Brown
P + Hydrogen peroxide (30%)	Green	Dark green	Fluorescent green	Pale green	Creamish	Creamish	Green	Orangish	Light orange

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3.4.2 Secondary metabolites

Alkaloids were in high amounts in chloroform and methanol extracts of all parts of *G. senegalensis*. The presence of alkaloids in the extract of *G. senegalensis* indicates that it may exhibit many pharmacological activities. Glycosides were also present in an appreciable quantity in the leaf and stem but not in the bark. Cyanogenetic and anthraquinone glycosides were present in higher amounts in methanol extracts of the leaf and stem. These are well-known laxative compounds that are beneficial for overcoming constipation. Anthraquinone glycosides have been reported to exhibit DNA-binding, anti-cancer, antimicrobial, cathartic, diuretic, and anti-inflammatory activities [27].

Furthermore, flavonoids were present in an appreciable amount in the leaf and stem and a moderate amount in the bark. They have several therapeutic biochemical and antioxidant effects that cure various illnesses such as Alzheimer's, atherosclerosis, cancer, etc. [28]. Flavonols and flavonones show antioxidant potential and reduced risk of vascular disease. These are found to be present in the leaf in high amounts. Phenolic compounds were also present in high quantities in all leaf and bark solvent extracts, whereas in moderate amounts in the stem. However, bioactive phenolic compounds were detected chiefly in bark and reported to act as metal chelators and radical scavengers.

Tannins are the most critical polyphenolic secondary metabolites, which show anti-aging, antimicrobial, antioxidant, and antiproliferative activities [29-31]. In the case of *G. senegalensis*, tannins were also present in traces in methanolic and aqueous extract of all the parts. Aqueous extract of leaf, stem, and bark of *G. senegalensis* also showed a higher presence of saponins. Terpenoids were present in petroleum ether extracts of all parts of G. senegalensis. It represents that petroleum ether extracts have the potential for a repair mechanism in favor of injuries and wounds; also, they can protect from environmental stress through chemical defense [32-34]. Steroids such as phytosterols and cholesterol, which show anti-inflammatory activity and immune-modulating properties, were also present in moderate amounts in the stem and bark and in traces in the leaf [35].

Gums have been widely used in the preparation of cough suppressing agents, coating agents for microcapsules, bulk laxatives for dental adhesives, emulsifiers, and as sustaining agents for buccal and matrix tablets for protein delivery [36]. In our study, the methanol and aqueous extracts of all parts of *G. senegalensis* showed the presence of gum in an appreciable amount. Acidic compounds which contain nitrogen and the amino group were not present in most of the solvent extracts from all parts of *G. senegalensis* except in the benzene extract of the leaf and the aqueous extract of the stem. Vitamin C was utterly absent in all solvent extracts of different plant parts. Organic acids such as oxalic acid, tartaric acid, and citric acid are known as fruit acids and were found in chloroform extracts of all parts of *G. senegalensis* in trace amounts. Malic acid was also present in trace and moderate amounts in all solvent extracts of different parts.

Based on the above discussion, petroleum ether and methanol extract of the leaf, stem, and bark of *G. senegalensis* were observed as rich resources of different types of bioactive compounds as compared to the other extracts. The results of the phytochemical screening are listed in **table 4**.

3.5 Element analysis by AAS

The lack of knowledge about the quantity and nature of elements present in the plant will pose an enormous threat to consumers as these plants may contain high concentrations of toxic elements. The presence of different elements and their amount in a plant depends on the water, fertilizers, and soil compositions. The lack of knowledge about the quantity and nature of elements present in the plant will pose an enormous threat to consumers as these plants may contain high concentrations of toxic elements.

Arsenic (As) can be found in airborne particles, soil, water, and salt. The dose of As can cause kidney, liver, skin, bladder, and lung cancer [37]. The current study observed 0.92 ppm in the leaf, 1.43 ppm in the stem, and 1.37 ppm in the bark of *G. senegalensis*. Chromium (Cr) is listed in the top 25 toxic, hazardous elements that pose a higher risk to the environment and human health. The World Health Organization (WHO), Central Pollution Control Board (CPCB, India), and US Environmental Protection Agency (USEPA) set the highest permissible limit (PL) at 0.1 and 0.05 mg/l, respectively [38-40]. All the samples (leaf, stem, and bark) were found in the range of 0.46, 1.04, and 0.03 ppm, respectively.

In different countries, cadmium (Cd) has gained more attention for exceeding the PL for medicinal plants and herbs given by the WHO and Food Agriculture Organization (FAO). At high concentrations, cadmium critically affects the vascular, liver, and immune systems [41]. In the present study, 0.04 ppm cadmium was found in the leaf, 0.02 ppm in the stem, and 0.16 ppm in the bark of *G. senegalensis*.

Lead exposure, at the early stage of pre-natal and childhood, has been linked with slow growth and development, learning difficulties, vision impairments, hearing, and much more adverse effects such as poor muscle organization in renal, nervous, reproductive, skeletal, cardiovascular, and muscular systems [42, 43]. It is well known, highly toxic environmental pollutant. In the present study, 0.43 ppm, 1.47 ppm, and 1.34 ppm of lead were found in the leaf, stem, and bark of *G. senegalensis*, which is significantly lower than the permissible limit (10 mg/kg) as suggested by FAO/WHO.

Among all, zinc (Zn) is an essential trace element necessary for the functioning of the thyroid, growth and development, blood clotting, and the synthesis of protein and DNA. High intake beyond the PL of 50 mg/kg can affect the levels of copper and blood lipoprotein in the human body [44, 45].

Table 4. Preliminary phytochemical screening of different parts (leaf, stem and bark) of *G. senegalensis*.

Plant constituents	Tests	Indication	leaf						stem						bark					
			PE	B	C	EA	M	AQ	PE	B	C	EA	M	AQ	PE	B	C	EA	M	AQ
Primary metabolites																				
Protein and Amino acids	Biuret test	Peptide bond	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ninhydrin test	Aliphatic primary amine and Amino acids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mulders's test	Aromatic amino acids, phenylalanine and tryptophan	-	+	+++	++	+	-	+	-	-	-	-	-	-	+	+	-	-	-
	Precipitation test	Secondary and tertiary structures of protein	-	-	-	-	++	-	++	+++	-	-	-	-	+	+++	-	-	-	-
	Millons Nasses test	Tyrosine and Xanthoproteic acid	-	++	-	-	+	-	-	-	-	++	++	+	-	-	-	-	-	-
	Nitroprusside test	Cysteine and sulphur containing amino acids	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Carbohydrates	Molisch's test	Pentose	-	-	+	++	+++	-	-	-	-	-	++	-	-	-	-	-	-	-
	Fehling's test	Reducing sugar	-	-	+	++	+++	-	-	+++	+	-	+	-	-	-	-	-	-	+++
	Iodine test	Starch, dextrin, and glycogen	++	-	+++	-	-	-	++	-	+++	-	-	-	++	+	+++	-	-	-
	Benedict test	Aldehyde and ketone	-	-	-	-	-	-	-	++	++	++	+++	++	++	-	+	-	-	-
	Barfoed's test	Monosaccharides	-	-	-	-	+	-	-	-	-	+	-	++	-	-	-	-	-	-
	Seliwanoff's test	Ketohexoses and fructose	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	Osazone test	Maltose and lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mucic acid test	Dicarboxylic acid and aldose sugar	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	+++	-	++	-
	Bial's orcinol test	Pentose and pentosans	++	+++	+	-	-	-	+	+	-	-	-	-	++	+	+	-	-	-
	Tollen's Phloroglucinol test	Galactose	+	-	+++	-	++	-	-	-	-	-	+++	-	++	+	+++	+	-	-
	Tannic acid test	Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lipids	Oil Stain test	Oil and fat	++	+++	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
	Saponification test		+	+	++	+	-	+++	-	-	-	-	-	+++	+	+	+	+	-	-
	Sudan red III	Essential oils and triglycerides	-	+++	+++	++	+	-	+	+++	+	++	-	-	++	+++	+++	++	+	-

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Table 4 continued…

Secondary metabolites																				
Alkaloids	Mayer's test	Cocaine, heroin, morphine, and nicotine	-	-	-	+	-	-	++	-	-	-	-	-	-	-	-	-	-	-
	Hager's test	Anhalonine	-	-	-	++	+++	-	++	-	++	+++	+++	-	-	-	-	-	-	-
	Wagner's test	Anhalonine , Quinoline and Nicotine	++	+	-	-	-	+++	++	++	++	+	+++	-	-	-	-	-	-	-
	Dragendroff's reagent test	Secondary and tertiary amines	+	+++	-	++	-	+	+++	+	+++	-	-	-	++	+	+++	-	-	-
	Bertrand's test	Caffeine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sonnenschein's test	Common alkaloid	+++	+	+	+	-	+	+++	+	-	-	+	+	+	+	++	-	+++	-
	Scheibler's reagent test	Choline	-	-	-	+++	-	-	-	+	-	+++	-	-	-	-	-	-	+++	-
	Murexide test	Purine alkaloids and caffeine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sodium carbonate test	Aconitine	-	-	-	+++	-	-	-	-	-	+++	-	-	-	-	-	-	+++	-
	Potassium ferricyanide	Brucine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sodium hydroxide	Coumarins	-	-	-	++	-	-	-	-	-	++	-	+	-	-	-	-	-	-
	Sodium phosphate	Heroine	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	Vitali-morin test	Solanaceous, tropane alkaloids, and Hyoscyamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Krauts' test	Anhalonine	-	-	+++	-	-	-	-	+++	-	-	-	-	-	-	-	-	+++	-
	Erdmann's test	Clofenotane, Mitotane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	Keller Killiani test	Cardiac glycosides, Bufadienolide and free sugar	+++	++	+	-	-	-	++	+	-	-	-	+	-	-	-	-	-	-
	Baljet reagent		+	+	+	+	++	+	+	++	+	-	-	-	+	++	-	-	-	-
	Mercurous nitrate test	Cyanogenetic glycosides	-	-	-	-	+++	-	-	-	-	+++	-	-	-	-	-	-	-	-
	Legal's test	Cardenolides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Hydroxyanthraq uinone test	Anthraquinone glycosides	-	-	-	-	+++	-	-	-	+	+++	++	-	-	-	-	-	-	-
Flavonoids	Alkaline reagents test	Common flavonoids	++	-	-	+	+++	-	+	+++	+	-	-	-	+	++	-	-	-	-
	Lead acetate test	Flavones	-	-	-	-	+++	-	-	-	-	+	++	-	+	-	-	-	-	-

Table 4 continued…

	Shinoda test	Xanthones, flavonones and flavononols	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	+++	+++	-
	Sulphuric acid test	Chalcones, Aurons, flavanes, flavones and flavonols	+	-	++	-	+++	-	+	+	++	+++	+++	-	++	+	+++	-	-	-
	Zinc HCL test	Common flavonoid	-	-	-	-	+++	-							-	-	-	-	-	-
Phenols	Potassium dichromate test	Aldehydes and ketones	++	++	++	++	+++	+++	+	+	+	+	+++	+	++	++	++	++	+++	+++
	Lead acetate test	Phenolic compound	-	-	+++	++	-	+	+	++	-	-	-	-	-	-	+++	++	+	+
	Folin-Ciocalteau test	Polyphenols	-	-	-	++	+++	-	-	-	+	-	-	-	-	-	-	-	-	-
Tannins	$FeCl_3$ test	Cathechic tannin	-	+++	-	-	-	-	-	++	+	-	-	-	-	-	-	-	-	-
	Bromine water	Condensed tannin	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	+	+
	Ammonia test	Chlorogenic acid	-	+++	-	-	++	-	-	+	-	-	+	-	+	-	-	-	-	-
	Dilute HNO_3 test	Phlobatannins	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	Hydrochloride test		-	-	-	-	-	-	-	-	-	+	++	-	-	-	-	-	-	-
Saponin	Olive oil test	saponin	-	-	-	-	++	+++	-	-	-	-	-	+++	-	-	-	-	-	+++
	Foam test		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Hemolysis test		-	-	-	-	-	+++	-	-	-	-	++	+++	-	-	-	-	-	+++
	Froth test		+	+	+	+	-	-	++	+++	-	+	-	-	+++	++	+	+	-	-
Terpenoids	Hirschhorn test	Triterpenoids	++	-	-	-	-	-	++	+++	-	-	-	-	+++	++	+	-	-	-
Steroids	Salkowski test	Phytosterols and cholesterol	+	-	+	-	+	-	-	-	+	++	+++	-	+	++	+++	-	-	-
Gums and Mucilage	Ruthenium red test	Mucilage	-	-	-	-	++	+++	-	-	-	-	+++	++	-	-	-	-	+++	++
Acidic compounds	Red litmus paper test	Nitrogen and Amino group	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Vitamins	Vitamin C	Vitamin C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic acids	$CaCl_2$ test	Oxalic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Silver mirror test	Tartaric and Citric acid	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
			-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
	$FeCl_3$ test	Malic acid	++	-	+	-	+++	-	+++	-	+	+	+	+	+	-	+	-	+++	+

- not present, + present in trace amount, ++ present in moderate amount, +++ present in high amount, PE: Petroleum ether, B: Benzene, C: Chloroform, EA: Ethyl acetate, M: Methanol, AQ: Aqueous

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In the current study, 0.59 ppm Zn was present in the leaf, 0.24 ppm in the stem, and 0.34 ppm in the bark of *G. senegalensis*.

Manganese (Mn) is an essential activator for enzymatic reactions with fats, carbohydrates, and protein metabolism. It is vital in transferring oxygen from the lungs to other human cells [46]. It is an essential nutrient, crucial for normal growth and metabolism in the body, despite many undesirable health effects at higher doses, such as impaired male fertility and congenital disabilities in offspring [47, 48]. The toxicity of manganese, however, occurs more often with iron deficiency. Also, Mn is considered safe and has not been classified as a carcinogen by International Agency for Research on Cancer (IARC) and WHO [49]. In the present study, 0.57 ppm Mn was reported in the leaf, 0.58 ppm in the stem, and 0.65 ppm in the bark of *G. senegalensis*.

On average, copper (Cu) is non-toxic to mammals [50]. Its deficiency could even consequence in weight loss, anemia, inflammation, demyelination of nerves, and other discomforts. An excessive Cu dose might cause serious health problems [51, 52]. Thus, this study screened the elemental composition of different parts (leaf, stem, and bark) of *G. senegalensis*. It revealed that it contains both the macro and microelements (As, Cr, Cd, Pb, Zn, Mn, and Cu), and their detected accuracy is calculated in parts per million (ppm) as given in **table 5**.

Table 5. Accuracy of macro and microelements in ppm determined by AAS.

Elements analyzed	Quantity of the element (ppm)		
	Leaf	Stem	Bark
As	0.92	1.43	1.37
Cr	0.46	1.04	0.03
Cd	0.04	0.02	0.16
Pb	0.43	1.47	1.34
Zn	0.59	0.24	0.34
Mn	0.57	0.58	0.65
Cu	0.33	0.49	0.24

4. Conclusion

In this research work, the pharmacognostic study of the leaf, stem, and bark of *G. senegalensis* has been carried out for the first time. Our results have standardized the physicochemical and phytochemical parameters of this medicinal plant. The present study revealed the presence of primary and secondary metabolites in the extracts of different parts of the plant. The stem and bark also showed a moderate presence of macro and microelements. This study justified the nutritional and ethnomedicinal benefits of *G. senegalensis* to human health and authenticated it as an herbal drug. A standard monograph will be prepared from our results that will be highly significant for the manufacturing of formulations of phytomedicine. This will also provide supplementary information to manufacturers, policymakers, consumers, and researchers to produce a good quality product of *G. senegalensis*.

Declarations

Author Contribution: Both authors contributed to the conception of the presented idea for the article; DJ did the literature search and performed experiments and data analysis; PJ validated the results, and both authors contributed to drafting the manuscript.

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