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Research Article

Investigating the Tannin Content and Phytochemical Composition of Water Hyacinth (*Eichhornia crassipes*) in Lake Tana, Ethiopia

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Nowadays water hyacinth coverage in Lake Tana is increasing day to day. Recently, considerable attention has been given to this plant to utilize and use as economical sources in many parts of the world. However, utilization of this plant is limited in Ethiopia. According to different findings, the tannin and other phytochemical contents of the water hyacinth vary from place to place where the plant grows up. Therefore, the aim of this study was to determine the tannin and other phytochemical constituents of the plant, in the case of Lake Tana, Ethiopia. The leaves and stem parts of the plant were extracted using distilled water, acetone, and ethanol solvents. The phytochemical, FTIR, and tannin content of the extracts were carried out following standard procedures. The phytochemical analysis revealed the existence of primary and secondary metabolites such as alkaloids, flavonoids, phenols, tannins, starch, and protein in the leaves and stems parts of the plant. The tannin content of water hyacinth leaves has higher tannin content as compared to that of the stem. During tannin content determination the total ash in % and pH value of leaf and stem tannins were determined. The pH of the leaf part was higher; however, the total ash content was lower than that of the stem. The FTIR spectrum data of the leaves and stems of the plant was recorded in the wavenumber range of 4000 and 500 cm^{-1}. This revealed the presence of different functional groups in both leaf and stem parts of the plant. The phytochemicals found in the plant parts, including primary and secondary metabolites, could be used for medicinal and pharmaceutical applications as well as for textile and tanning industry wastewater treatment. Similarly, according to the findings of this study, water hyacinth leaves and stem parts have a tannin which will be used as an ideal agent for the tanning and retanning process in leather processing.

Keywords: extracts water hyacinth; FTIR; leaves; metabolites; phytochemical analysis; stems

1. Introduction

The free-floating aquatic plant water hyacinth (*Eichhornia crassipes*) is known to cause significant ecological and socio-economic changes [1]. Water hyacinth is now considered one of the world's worst invasive plants, causing trouble for millions of people who depend on water resources. According to the International Union for Conservation of Nature (IUCN), the plant is among the Top 100 most harmful invasive species and the Top 10 worst weeds in the world [2]. The water hyacinth plant comes in seven different species, but a few popular ones include *Eichhornia crassipes*. It

is often considered a weed, but it has been used in a variety of tests, including the manufacture of ethanol [3]; as an adsorbent for heavy metals found in wastewater [4]; for phytoremediation [5]; as a raw material for the production of biogas [6]; as a biofuel [7]; and as a protein supplement in ruminant feed [8]. Currently, water hyacinth is infesting Lake Tana, Ethiopia. Lake Tana is Ethiopia's largest lake and the Blue Nile's second largest subbasin, with a surface area of 15,114 km^2 [9]. Fishing, electric power generation, transportation, common grazing land and drinking for humans and animals, and a site for various birds are some of the multidisciplinary uses of Lake Tana [10]. However, this multipurpose lake is currently plagued by several issues.

As shown in Figure 1, the Tana biosphere on the shores of this sensitive Lake Tana, where water hyacinth is one of the most ecologically dangerous weed infestations, has recently been reported [11, 12]. Nearly 35 invasive alien plant species are causing negative impacts on Ethiopia's natural biodiversity [13]. Water hyacinth was first discovered in Ethiopia in 1956 in Koka Lake and the Awash River and has been known as the country's most harmful aquatic weed since 1965 [12, 14]. However, the existence of the plant in the Lake Tana has been recognized since 2011, and the area covered at the time of inception was approximately 80–100 ha [15, 16]. Its coverage continues to escalate from 20 ha in 2012 to more than 50,000 ha in 2014 [15]. Even if a tremendous amount of human labor, time, and money has been exerted each year by both the surrounding community and the government, the infestation coverage continues to escalate in the range of 278.3 and 2504.5 ha from 2015 to 2019 [11]. Even if both the local people and the government put in a considerable effort and resources per year, the infestation coverage continues to rise in the range of 278.3 and 2504.5 ha from 2015 to 2019 [17]. This aquatic plant has recently received a lot of attention as a potential source of income in many parts of the world [18, 19]. However, there are very few reports on the utilization of water hyacinth in Ethiopia. According to Jayanthi and Lalitha [20], the tannin and related contents of the water hyacinth plants vary from place to place where the plant grows. In their study, the tannin was not found in water hyacinth collected from lakes from Tikitpur village and Kurichi in Coimbatore. However, Goud et al. [21] reported a higher tannin content in *Eichhornia crassipes* (WH) collected from Tondano Lake in the district of Minahasa, North Sulawesi Province, and Warangal district, Andhra Pradesh, respectively. It is revealed that the possible reasons for these variations are because the plant needs sunlight, water, carbon dioxide, and nutrients, including phosphorous, nitrogen, and potassium, to grow which varies from water body to water body [22, 23]. The present study was conducted to investigate the presence of tannin content in water hyacinth plant stems and leaves collected from Tana Lake in Ethiopia. Similarly, it was aimed at detecting the presence of phytochemicals in the plant parts. Furthermore, it was targeted to examine the functional groups available in the plant for leather tanning and industrial wastewater treatment. The tannin content of water hyacinth leaves and stems was determined using the hide powder process based on the following literature. The research work has been carried out at the laboratory available in the Ethiopian Institute of Textile and Fashion Technology, Bahir Dar University, Ethiopia.

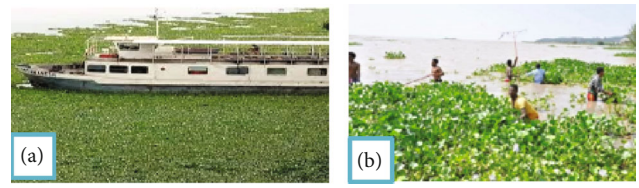


Figure 1: (a, b) Water hyacinth infestation in Lake Tana (http://www.africanewschannel.org).

2. Materials and Methods

2.1. Materials

2.1.1. Plant Collection. The leaves and stems of healthy water hyacinths were collected from Lake Tana, near St. Kirstos Samra Monastery in Gonder district, Ethiopia, as shown in Figure 2.

The collected plant leaves and stem parts were cleaned and washed with water and cut into small chips for faster drying. The leaves and stems were dried outdoors for 7 days at temperatures ranging from 25°C to 30°C and then oven-dried for 1 h at 104°C.

2.2. Plant Powder Preparation. The dried leaves and stems were individually weighed and ground in a high-speed multifunctional grinder with a mesh size of 50–300 mesh. The fundamental idea behind grounding the plant part was to maximize the surface area available for extraction, thereby raising the extraction rate [24]. The powdered samples are placed in small plastic bags for phytochemical analysis and tannin content determination.

2.3. Solvents Used for Extraction. The types of solvent used in the extraction process have a big impact on determining biologically active compounds from plant content [25]. The quantity of phytochemicals to be extracted, the rate of extraction, the diversity of different compounds extracted, the diversity of compounds extracted, the ease of subsequent extract handling, and the toxicity of the solvent in the bioassay process are all considerations that influence the solvent selection [26]. According to the studies of Tyagi and Agarwal [27] and Ahmed et al. [28], the phytochemical study of leaves and stem extract of plants was conducted using distilled water, ethanol, and acetone solvents.

2.4. Preparation of Aqueous and Solvent Extracts. The leaves and stems of the water hyacinth were chosen for phytochemical analysis. For extract preparation, three different solvents (distilled water, acetone, and ethanol) were used, as shown in Table 1.

Fifty grams of leaves and 50 g of stem powders were extracted separately in 500 mL of distilled water, acetone, and ethanol solvents by maceration process [29]. The extract was then put in a glass beaker with aluminum foil on top and left to stand at room temperature for 48 h. The mixtures

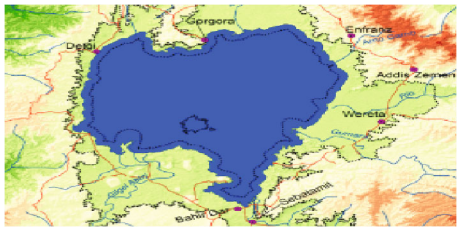


FIGURE 2: Sampling collection site, around St. Kirstos Samra Monastery in Lake Tana, Ethiopia.

were also purified for 10 min at room temperature using a centrifuge 5810 with 4000 rpm centrifugation at room temperature. The extracts were then preserved in volumetric flasks in a refrigerator at 20°C, for phytochemical analysis.

2.5. Qualitative Phytonutrients Tests and Characterization. Phytochemicals are nonnutritive plant chemicals that have disease-preventive properties. These chemicals are generated by plants to protect themselves, but study shows that certain phytochemicals can also protect humans from disease. Steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, and glycosides are examples of plant bioactive constituents [30]. The prepared extract was subjected to preliminary qualitative phytochemical screening for alkaloids, flavonoids, tannins, saponins, collagen, and phenol analysis following standard test methods.

Detection of flavonoids using alkaline reagent test method: A small amount of extract was treated with an aqueous NaOH solution which leads to the formation of yellow–orange color [31].

Detection of tannin using ferric chloride test method: In a test tube, a 2-mL portion of the extract was placed, and the extracts were treated with a few drops of 10% ferric chloride solution and observed for the formation of a blue or greenish color [32, 33]. Detection of saponins using froth test method: A 0.5-g of extracts was mixed with 2 mL of water and shaken for 15 min in a graduated cylinder. The presence of saponins is shown by the formation of a 1-cm layer of foam [34]. Test for alkaloids using Mayer's test method: Mayer's reagent test was used to process the extracts (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 mL of water). The presence of alkaloids is indicated by the formation of a yellow-colored precipitate [35]. Detection of phenols using the ferric chloride test: 3–4 drops of 5% ferric chloride solution were added to the extracts. The presence of phenols is indicated by the formation of a bluish–black color [36]. Detection of starch using starch reagent: The aqueous extract (5 mL) was treated with the starch reagent (iodine). The presence of starch is identified if the blue–violet color is observed [37]. The color change shown in the starch detection experiment of this study is shown in Table 2. Detection of protein using the Biuret test: Two drops of 1% copper sulphate solution is applied to a 0.5-mg extract equivalent amount of 40% NaOH solution [38]. The appearance of protein is indicated by the formation of a violet color, and the color change is indicated in Table 2.

2.6. Quantitative Phytochemical Test. Quantification of tannin would help to know the amount of tannin content present in the water hyacinth leaves and stems parts. The tannin content of plant leaves and stems was determined using the hide powder process, and the tannin content was determined at the Central Leather Research Institute (CSIR) Laboratory, India. The tannin content results of the present study were compared with the findings of other studies which is presented in Table 2.

3. Result and Discussion

As shown in Table 1, the detection results of the phytochemicals are indicated with different signs. A strong positive test is indicated with (++), a weak positive test is indicated with (+), and while not detected it is indicated with (−). These signs are given based on the subjective evaluation; if the color maintained at its standard detection method is seen in deep bright, we call it a strong positive test; on the contrary, if the indicated color is not observed, the phytochemical is not detected (−), and its shallow color indicates a weak positive test. Plant secondary metabolites protect it from predation by a variety of microorganisms, insects, and herbivores [39].

As shown in Table 1, flavonoids, saponins, phenols, tannins, alkaloids, starch, and proteins were found in distilled water, ethanol, and acetone extracts of water hyacinth plant leaves and stems. This indicates the plant will have medicinal properties due to its phytochemicals [40]. Flavonoid detection result: Flavonoids are a large family of polyphenolic plant compounds. There are six major subclasses of flavonoids, namely, anthocyanidins, flavan-3-ols, flavanones, flavones, and flavones. Among these, the current study found strong positive (++) flavonoids in distilled water and ethanol extracts of the stems, and weak positive (+) flavonoids were detected in distilled water and acetone extracts of the plants. Relative better results were found compared to other researchers [20, 41] who revealed flavonoids in water hyacinth collected from Singanallur boat house, Coimbatore, Tamil Nadu, and Tondano Lake in the district of Minahasa, North Sulawesi Province.

Alkaloids detection result: Alkaloids are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure may also be termed alkaloids. The presence of strong positive (++) alkaloids was indicated in the distilled water leaves extract with deep yellow color participation. The presence of weak positive (+) alkaloids was indicated in both leaves and stems acetone and ethanol extracts. Related findings by [42] and Lata [43] showed the existence of alkaloids in *Eichhornia crassipes* (WH) collected from lakes near Kurichi in Coimbatore, India, and Bilaspur district of Chhattisgarh, India. Based on this study and literature, due to polarity differences, solvents have a great effect on the detection of the phytochemicals in the plant.

Saponins detection result: Saponins are naturally occurring compounds that are widely distributed in all cells of

TABLE 1: Result of phytonutrient analysis of the WH plant leaves and stem.

No	Compound	Distilled water extract (leaves)	Distilled water extract (stems)	Ethanol extract (leaves)	Ethanol extract (stems)	Acetone extract (leaves)	Acetone extract (stems)
1	Flavonoids	+	++	−	++	+	+
2	Saponins	++	++	+	−	+	−
3	Phenols	++	++	+	++	++	++
4	Tannins	++	++	+	+	++	++
5	Alkaloids	++	+	+	+	+	−
6	Starch	++	+	+	++	−	++
7	Protein	++	+	+	+	+	−

Note: Key: strong positive test (++), weak positive test (+), and no detection (−).

TABLE 2: Comparison of tannin contents in the leaves and stems of the water hyacinth plant.

No.	Species	Plant part	Tannin content (%)	Plant property Total ash (%)	PH water soluble	Test method
1	Water hyacinth	Leaves	4.1	14.8	6.3	IS 5466: 2013
2	Water hyacinth	Stems	2.7	15.6	5.8	IS 5466: 2013

legume plants. Froth test revealed the existence of a strong positive (++) saponins in distilled water from both stems and leaves, as well as weak positive (+) saponins in ethanol and acetone extracts. Similar results were found by related researchers [44] who revealed saponin presence in *Eichhornia crassipes* (WH) collected from various marshy lands in Kayamkulam, Mavelikkara, Allepy, and Kerala. However, according to Lalitha et al. [42], saponin was not detected in *Eichhornia crassipes* (WH) obtained from lakes near Kurichi in Coimbatore, India. Phenol detection result: Phenols are a class of organic compounds containing a hydroxyl group and a benzene ring, the simplest of which is referred to as phenol. This study revealed the presence of strong positive (++) phenols in both stems and leaves in distilled water and ethanol extracts, strong positive (++) phenols were detected in stem acetone extracts, and weak positive (+) phenols were detected in acetone leaf extracts. Similar findings were reported by Jayanthi and Lalitha [20, 41], who reported the presence of phenols in *Eichhornia crassipes* (WH) collected from Singanallur boat house, Coimbatore, Tamil Nadu, and Tondano Lake in the district of Minahasa, North Sulawesi Province, respectively.

Starch detection result: Starch is a chain of glucose molecules that are bound together, to form a bigger molecule, which is called a polysaccharide. There are two types of polysaccharides in starch: amylose—a linear chain of glucose. As indicated in Table 1, the presence of strong positive (++) starch was detected in both acetone and ethanol stem extracts. Also, a weak positive (+) starch was detected in both distilled water extracts and ethanol extracts of the leaves. The results clearly indicate that the extraction solvent greatly affects the detection of starch in the plant.

Tannin detection results: Tannins are amorphous, astringent compounds found in a broad range of plant materials, including bark, wood, leaves, and resinous exudations [45], and they are polymeric phenolic compounds with various hydroxyl groups and chemical structures that are very complex [46]. In this study, strong positive (++) tannins were detected in both stems and leaves in distilled water extracts and acetone extracts, and weak positive (+) tannins were detected in both ethanol extracts of leaves and stems.

Protein detection results: A protein is naturally occurring and is a complex substance that contains amino acid residues joined by peptide bonds. It can be found in all living organisms with including biological compounds such as enzymes, hormones, and antibodies. The Biuret test revealed the presence of a strong positive (++) protein in the leaves distilled water extract, and a weak positive (+) protein was observed in both ethanol and acetone leaves and stem extracts.

As indicated in Table 2, tannin was present in both parts of the plant. The amount of tannin in the leaves is higher (4.1%) than that of the water hyacinth stem's part (2.7%). One of the possible reasons for the difference in tannin and other substance content is because the plant stem and leaf parts have different maturity and structural natures. In addition, the water hyacinth's tannin content is greatly influenced by its morphology, especially its leaf size and stage of development; leaves typically contain more tannin than stems, and environmental factors like nutrient availability and water quality can also affect the concentration of tannins in various plant parts; in general, older, larger leaves cultivated under nutrient-rich conditions have a tendency to have higher tannin content than younger or nutrient-poor leaves [47]. The ecological, morphological, developmental, and biological characteristics of water and its ease of growth stimulation in the presence of nutrients, particularly high concentrations of nitrate and phosphate, account for the difference [48]. Though metabolites also affect the maturities of leaves and stem parts, the solvent used to extract it has a great influence to quantify the difference between the leaves and stems [49]. The phytochemical analysis of tannins and

TABLE 3: Infrared spectroscopy summary table. Both water hyacinth leaves and stems were subjected to FTIR analysis in order to determine the available functional groups in the plants.

WH leaves	WH stem	Functional group assignment	Discussion of compound class	Reference
3340 cm^{-1}	3436 cm^{-1}	O-H stretching	Because of the presence of cellulose, the peak at 3328 cm^{-1} is due to alcohol/carboxylic acid O-H stretching.	[59]
2890 cm^{-1}	2889 cm^{-1-}	C-H stretching	It has been established that the 3000–2800 cm^{-1} region primarily represents the CH_3 and CH_2 lipid groups. Since the asymmetric stretch of the CH_3 group causes absorption in the 2990–2945 cm^{-1} region	[60, 61]
2346 cm^{-1}	2347 cm^{-1}	C≡C stretching	The presence of the C-C alkynes group is indicated by the peak at 2342 cm^{-1}. Additionally, amino acids and phosphorus compounds are represented by bands near 2342 and 2360 cm^{-1}.	[62]
1640 cm^{-1}	1634 cm^{-1}	C=O stretching	The peak at 1640 cm^{-1} was thought to come from C=O vibrations of carboxylates in a study on organic matter decomposition, and the peak at 1640 cm^{-1} reflects carboxyl group absorption.	[63–65]
1609 cm^{-1}		C=C stretching	The C-C aromatic stretching with strong conjugated C-C bond is indicated by the peak at band 1609 cm^{-1}, which is due to lignin material.	[66]
1318 cm^{-1}	1317.7 cm^{-1}	O-C=O stretching	The peak at 1318 cm^{-1} is due to the O-C=O asymmetrical stretching.	[67]
—	1030 cm^{-1}	C-O stretching	Primary alcohol C-O stretching in cellulose and hemicelluloses. The existence of alcoholic hydroxyl groups is indicated by the presence of C-O stretch.	[65, 68, 69]

phenols indicated that both stem and leaf extracts have a strong positive result almost in all of the solvents. The presence of different phytochemicals in the water hyacinth indicates the possibility of utilizing the plant for tanning and wastewater treatment. As shown in Table 2, the tannin content of water hyacinth leaves (4.1%) is relatively similar to *Salix folium* 4.0%, banana bunch of Kepok 4.1% [50], and Acacia senegal barks 3.49% [51]. However, according to Jayanthi and Lalitha [20], the tannin was not present in the water hyacinth obtained from Tikitpur village and the lakes near Kurichi in Coimbatore. This indicates the amount of tannin present in the plant can be affected by the place where the plant grew up and the solvents used during extraction.

3.1. Different Factors Affecting the Potential Metabolites Yields and Applications of Water Hyacinth Plants. Processing costs involve all expenditures related to the extraction, purification, and production of metabolites, which could be affected by raw materials accessibility; that means if it can be accessed easily in massive amounts, its cost will be relatively lower. Energy consumption: Some production methods are energy-intensive, especially bioprocesses that require continuous heating, cooling, or aeration. Labor: Labor costs vary depending on the complexity of the process. For example, fermentation processes may require skilled labor for monitoring and control, and if the water hyacinth is disposed of, biotechnological processes often generate by-products that need to be treated or disposed of, contributing to additional costs related to technological aspects: the effect of fermentation and enzyme catalysis. Cost-effectiveness of water hyacinth at large scales: the production of ethanol can be quite cost-effective due to high throughput, even though raw materials and energy requirements are still costly; however, it could be competitive if there are excess raw materials, cheap labor, and land costs. However, still, the mass industrial use of water hyacinth can present several environmental risks [52–55].

3.2. Potential Applications and Environmental Concerns of Massive Water Hyacinth Harve Station. Large-scale tannin and other metabolite extraction from water hyacinth (*Eichhornia crassipes*) entails a number of stages that can be tailored according to the metabolite type and extraction scale; to apply it for leather and other industries, levels are affected by different factors; it can be used for valuable products like leather tannin, fertilizer, or biofuel [19]. Monitoring water quality after harvesting and involving local communities in sustainable management practices are some potential solutions to lessen the environmental effects of water hyacinth harvesting. In other words, transform the "problem" of invasive hyacinth into a resource by figuring out how to use it while minimizing detrimental effects on the aquatic environment [56–58].

3.3. FTIR Analysis. In order to identify the functional groups available in the plant an important instrument, Fourier transform infrared spectroscopy [70, 71] was used, and the results are presented in Table 3. The leaves and stems of the water hyacinth plant were analyzed using FTIR to determine the plant's relative functional group of leaves and stems. The observed IR spectra were recorded using a Perkin Elmer FTIR Spectrophotometer in the range of 4000–400 cm^{-1} [72]. As shown in Figures 3 and 4, the spectra of leaves and stems are almost the same at finger print region. However, the characteristic bands of each functional group

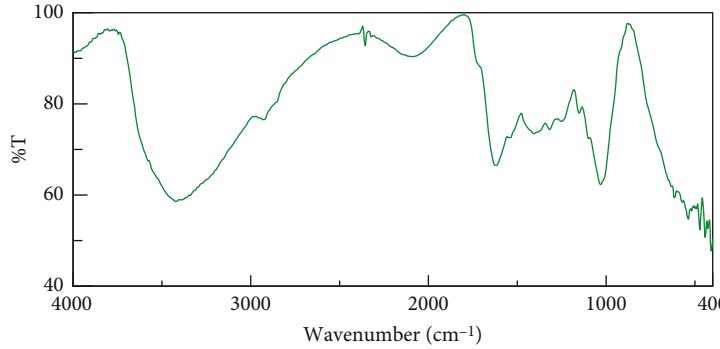


Figure 3: FTIR spectrum of water hyacinth leaves.

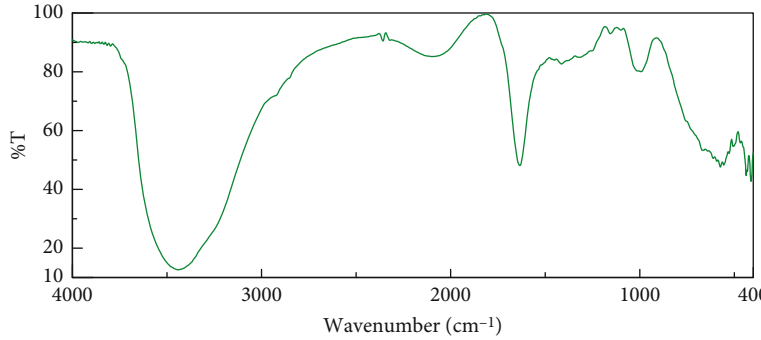


Figure 4: FTIR spectrum of water hyacinth stems.

for both stem and leaves are quite different. The spectra of both steam and leaves of water hyacinth contain so many functional groups that they will probably form multifunctional compounds.

As shown in Figure 3, water hyacinth leaves exhibited the presence of carboxylic acid/alcohol (O–H stretching), which was revealed by the very broad peak between 3340 and 2500 cm^{-1} is more probably due to carboxylic acid. The presence of alkanes/lipids (sp^3 C–H stretching) was revealed by the peak to the right of 3000 cm^{-1} around 2890 cm^{-1}. The alkynes have bands at 2346 and 2347 cm^{-1} (sp C≡C stretching). The peak at 1640 cm^{-1} revealed carboxyl group absorption (C=O stretch) and the presence of lignin content at 1609 cm^{-1} (sp^2 C=C stretch). The O-C=O asymmetrical stretching caused the peak at 1318 cm^{-1}, and a peak at 1063 cm^{-1} showed the presence of hemicellulose (C-C stretching). As shown in Figure 4, water hyacinth stems exhibited the presence of a sharp, strong, and broad peak around 3436 cm^{-1} assigned for alcohol (O–H stretching). Additionally, sp^3 C–H stretching is very common for all spectra and weakly appeared around 2889 cm^{-1}. The carbonyl functional group (C=O stretch) also appears around 1634 cm^{-1} The absorption bands of lignin, cellulose, and hemicelluloses are well known to be in the wavelength range of 1800–800 cm^{-1} (stretching and bending vibrations within the molecules. A peak of 1030 cm^{-1} revealed C–O stretching of primary alcohol in cellulose and hemicelluloses, and it is a confirmatory test for the presence of an alcohol functional group [73].

4. Conclusion

In water hyacinth leaves and stem extracts, phytochemical analysis revealed the existence of primary and secondary metabolites such as alkaloids, flavonoids, phenols, tannins, starch, and protein. The contents of phytochemicals in the plant parts were highly affected by the type of solvents used for extraction. The flavonoid compound of the leaf extracts was not detected using ethanol solvents; however, in the stem part, a strong positive flavonoid was detected. The phytochemical analysis revealed the presence of a strong positive tannin compound on both parts of the plant. Alkaloids, starch, and saponins of the stem parts of the plant were not detected using acetone extraction solvents. The tannin content of water hyacinth leaves and stems was determined using the hide powder process, with the plant leaves having the higher tannin content (4.1%) compared to the plant stems (2.7%). The pH of the tannin extracted from the leaf was 6.3, and that of the stem was 5.8. However, the total ash of the stem is found higher (15.6) than that of the stem (14.8). In both the leaves and stems of the plant, FTIR tests showed the presence of carboxylic acid (O–H stretch), alkanes/lipids (C–H stretch), and alkynes (C-C stretch) functional groups. Water hyacinth plant has recently got attention as a way to utilize as an income source. This study revealed the presence of various phytochemicals in the plant leaves and stem parts that could be used for pharmaceutical applications and wastewater treatment. The presence of

tannin in the plant could be used for leather tanning and retanning process in leather processing. Utilizing water hyacinth for different applications will reduce the effect of the plant on Lake Tana, Ethiopia.

Data Availability Statement

All data collected and analyzed during this study is included in the paper and can be accessed from the corresponding author with the rational request.

Conflicts of Interest

The authors declare no conflicts of interest.

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