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Phytochemical Profiling and FTIR Analysis of Semi-polar Extracts from *Digitaria horizontalis* Aerial Parts

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Abstract

Digitaria horizontalis is a grass species commonly found in tropical and subtropical regions. In traditional medicine, it is used as a local antibiotic to treat various ailments such as wound infections, inflammatory diseases, and gastrointestinal disorders. The study aims to investigate the phytochemical composition and characterize the functional groups present in the semi-polar extracts of *D. horizontalis* aerial parts through phytochemical profiling and Fourier Transform Infrared (FTIR) spectroscopy. The plant materials were shade-dried and pulverized to powder and cold macerated with chloroform and ethyl acetate each for 48 hours to afford chloroform and ethylacetate extracts respectively. Phytochemical screening was conducted on the extracts using standard procedures. Preparatory TLC was conducted on the extracts using the appropriate solvent system. FTIR (4000-650 cm^{-1}) analyses of chloroform extract (CE) and ethyl acetate extract (EAE) were conducted by dissolving 10 mg of the samples in 0.5 cm^3 deuterated solvents. The percentage yield of the extracts shows that EAE has the highest recovery (5.57%) and the CE (4.48%). Phytochemical screening conducted on CE and EAE indicated the presence of saponins, cardiac glycosides, carbohydrates, tannins, flavonoids, alkaloids, anthraquinones, steroids, and coumarins. FTIR analysis provided insight into the functional groups present (–NH, -CH, $-CH_3$. –C=O, C-C, C=C, -OH and –NO) in the extracts, aiding in the compound identification. In conclusion, the aerial parts of *D. horizontalis* contain potential phytoconstituents with major functional groups of alcohol, aldehyde, carbonyl compounds, amines, phenol, and nitro and thus should be studied further for possible isolation.

Keywords: Thin layer chromatography, *Digitaria horizontalis*, Retention Factor, Phytochemical screenings.

Introduction

Digitaria species were the earliest domesticated plants for human food, although they were eventually replaced by other species [1]. The *Digitaria* genus contains over 300 species, however the most frequent weedy species include *Digitaria ciliaris,* (crabgrass) *Digitaria ischaemum*, and *Digitaria sanguinalis.* While the species have diverse morphologies, their biology is almost identical. Herbicides are often employed to control *Digitaria* spp., however biological,

cultural, and mechanical methods can be used to effectively control plants in disturbed regions [2]. *Digitaria horizontalis* is native to tropical and subtropical regions, and it has a wide distribution across different continents. It can be found in parts of Africa, Asia, the Americas, and Australia. The entire parts of this plant, including the leaves, stems, and seeds, can have various uses. In traditional medicine, some cultures in northern Nigeria use *Digitaria horizontalis* for its medicinal properties, such as in the treatment of digestive disorders and skin ailments [3].

Literature shows that, the species exhibit differential phenotypes, the biology and management is nearly identical between the species [4]. However, herbicides are commonly used to control *Digitaria* spp., biological, cultural, and mechanical tactics can be utilized to effectively control plants inhabiting the disturbed areas [2]. The evaluation of the phytochemical or phytoconstituents of *Digitaria horizontalis* can reveal potential bioactive compounds with pharmacological properties, contributing to the development of new therapeutic agent [4]. This study aim to investigate the phytochemical composition and characterize the functional groups present in the semi-polar extracts of *D. horizontalis* aerial parts through phytochemical profiling and Fourier Transform Infrared (FTIR) spectroscopy.

Materials and Methods

Sample Collection, Identification and Preparation

The fresh samples of *D. horizontalis* were collected from rice farm at the Tony Elumelu Bridge, Usman Danfodiyo University Sokoto, Wamako Local Government Area of Sokoto State in the month of August 2024. The plant materials were identified and authenticated at the herbarium of Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The specimen was prepared and voucher number UDUH/ANS/0889 was issued. Fresh leaves of *D. horizontalis* aerial parts were properly washed to remove earthly impurities. The leaves were shade air-dried and pulverized to powder using a wooden pestle and mortar. The powdered sample was then stored in an air-tight container until it was needed for analysis.

Extraction Procedure

The powdered samples (200.00 g) were sequentially extracted with chloroform and ethyl acetate each for 48h with occasional shakings. The mixture was then filtered twice with muslin cloth and then with Whatmann No. 1 filter paper. The filtrates were then evaporated to dryness using rotary evaporator at 45^0C to afford chloroform and ethyl acetate crude extracts and was labelled as CE and EAE respectively [5].

Qualitative Phytochemical Study

Chloroform (CE) and ethyl acetate (EAE) extracts from the aerial part of *D. horizontalis* were subjected to phytochemical screening using the standard procedure [6][7].

Thin Layer Chromatography and Preparatory TLC

Thin-layer chromatography (TLC) was conducted on the plant's extracts using TLC pre-coated plates (silica gel 60F254) by using one-way ascending technique as described by Arnason *et al.* [8] and Yusuf *et al.,* [9]. In this method, pre-coated TLC plates were cut with a scissor and marked with a pencil about 1 cm from the bottom of the plate. Each sample (extracts and fractions) was faintly dissolved in appropriate solvent and capillary tubes were used to uniformly apply the dissolved samples on the plates and allowed to dry. The plates were developed in a chromatographic tank using the different solvent systems at room temperature. Preliminary TLC separations of all the extracts were carried out using (EA/CF 8:2 and EA/ME 9:1). The developed chromatograms were air dried and visualized under ultraviolet light (254 and 365 nm) and by spraying with 10% sulphuric acid in water followed by heating at 105 ^{o}C for 5-10 minutes in an oven. The R_f values of distinct components were calculated using the formula in the equation

$$Rf = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}} \ldots\ldots\ldots\ldots\ldots Eq\ 1$$

FTIR Analysis of CE and EAE: The FTIR (4000-650 cm^{-1}) analysis of chloroform and ethyl acetate of *D. horizontalis*was conducted by dissolving 10 mg of the isolate in 0.5 cm^3 deuterated chloroform ($CDCl_3$). The transmittance method was used with resolution 8 to identify the type of functional groups present.

Result and Discussion

Results

Extraction yield of the *D. horizontalis* extracts

The sequential extraction of 200.00 g of *D. horizontalis* afforded the following percentage yields as presented in Table 1. The ethyl acetate extract was found to have the highest percentage yield of 5.71 %.

Table 1: Percentage yield of *D. horizontalis* Extracts

Extract	Yield (%)	Nature of the Extract	Colour of Extract
Chloroform	4.48	Solid	Green
Ethyl acetate	5.71	Solid	Green

Phytochemical Screening

Preliminary phytochemical screenings performed on chloroform and ethyl acetate extracts of *D. horizontalis* indicated the presences of alkaloids, flavonoid tannins, steroids/triterpenoids,

saponins, glycosides, cardiac glycosides, Coumarins, Carbohydrate, and anthraquinones, which exhibited variations among the extracts (Table 2 and 3).

Table 2: Preliminary Phytochemical Screening of *D. horizontalis* CE

Constituents	**Test**	**Observation**	**Inferences**
Carbohydrates	a. Molisch's test b. Fehling's test	Violet ring Red ppt	- -
Phenoles	a. Feric chloride test	Greenish black colour	-
Flavonoids	a. Feric chloride test b. Shinoda's test c. NaOH	Greenish black colour Pink or red colour Yellow colour	- - -
Tannis	a. Feric chloride test b. Lead acetate test	Greenish black/blue Coloured ppt	- -
Alkaloids	a.Mayer's test	Milky or yellow ppt	-
Antraquinones	a. Borntrager's test	Bright/cherry red	-
Steroids and terpenoids	a. Salkowki's test b. Lieberman-Buchard's test	cherry red/golden bra reddish/brawn ring	+ +
Saponins	a. Frothing test	Foam	-
Cardiac glycoside	a. Killiar-Killiani's	Purple – brawn	+

Key: + = presence and - = absence

Table 3: Preliminary Phytochemical Screening of *D. horizontalis* EAE

Constituents	**Test**	**Observation**	**Inferences**
Carbohydrates	a. Molisch's test b. Fehling's test	Violet ring Red ppt	+ +
Phenoles	a. Feric chloride test	Greenish black colour	-
Flavonoids	a. Feric chloride test b. Shinoda's test c. NaOH	Greenish black colour Pink or red colour Yellow colour	+ + +
Tannis	a. Feric chloride test b. Lead acetate test	Greenish black/blue Coloured ppt	+ +

Alkaloids	a.Mayer's test	Milky or yellow ppt	-
Antraquinones	a. Borntrager's test	Bright/cherry red	-
Steroids and terpenoids	a. Salkowki's test b. Lieberman-Buchard's test	Cherry red/golden bra Reddish/brawn ring	+ +
Saponins	a. Frothing test	Foam	-
Cardiac glycoside	a. Killiar-Killiani's	Purple – brawn	+

Key: + = presence - = absence

Thin Layer Chromatography (TLC) of *D. horizontalis* of Extracts

Preliminary TLC profiles of chloroform and ethyl acetate extracts of *D. horizontalis* viewed under 254 nm, 365 nm and after spraying with 10 % sulphuric acid and heating in an oven at 105 ^{0}C using solvent system (EA/CF 8:2 and EA/ME 9:1). TLC analysis of the extracts revealed the constituents separation using different solvent systems and the plate are presented below (Plate 1 and 2). Some compounds from the TLC are UV active while others are not.

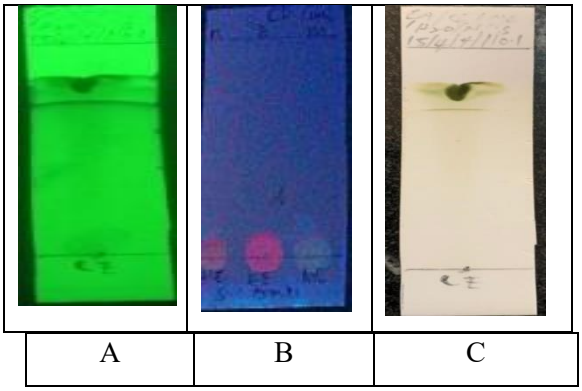

Plate 1: TLC profile of chloroform extract of *D. horizontalis* using EA/CF 8:2 and EA/ME 9:1as Solvent System; (A) at 254 nm (B) at 365 nm (C) after heating

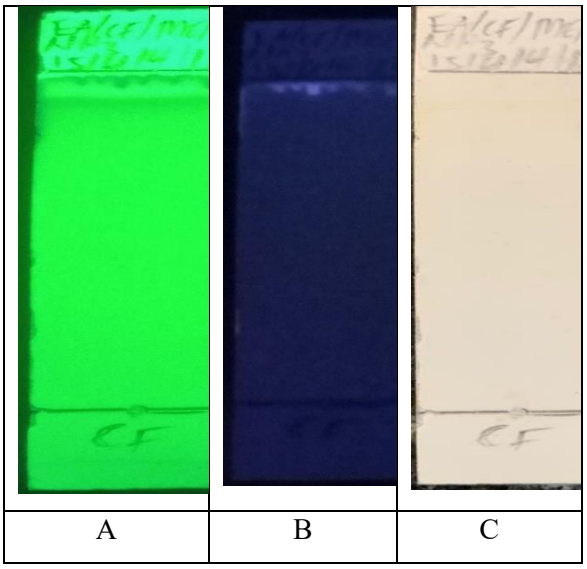

Plate 2: TLC profile of ethyl acetate extract of *D. horizontalis* using EA/CF 8:2 and EA/ME 9:1as Solvent System; (A) at 254 nm (B) at 365 nm (C) after heating

Table 4: Rf Values of the distinct Components of *D. horizontalis* aerial part extracts

Spots	CE	EAE
A	0.72	0.98
B	0.80*	0.71
C	0.83	0.64
D		0.21*

Key: CE = Chloroform extract, EAE = Ethyl acetate extract and * = UV Active

Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The FTIR analysis for the identification of the principal functional groups of *D. horizontalis* extracts and fractions were presented in Table 4-7.

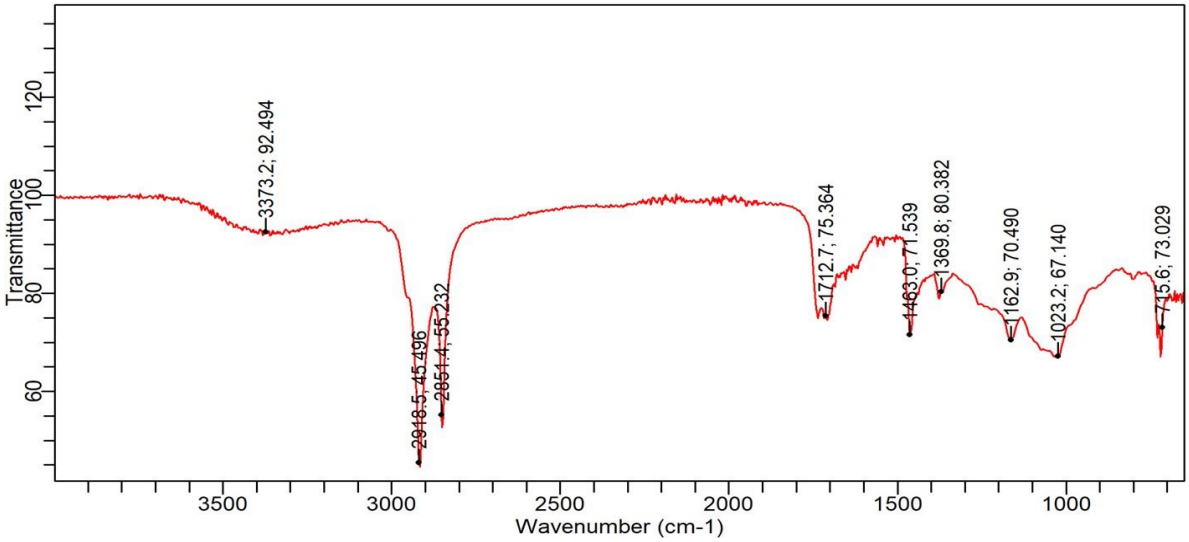

Figure 1: FTIR Analysis of *D. horizontalis* chloroform extract

Table 5: FTIR data of *D. horizontalis* chloroform Extract

Band	υ (cm^{-1})	*υ (cm^{-1})	Inference
O-H alcohol	3373.2	3250-3450	O-H stretching
C-H of aldehyde or carbonyl	2918.5	2700-3000	C-H stretch alkenes, carboxylic acid and alcohol
C-H of alkane	2851.4	2031	C-H stetching
C=O of carbonyl	1712.7	1700-1750	C=O stretch esters
C-H of alkane	1463.0	850-880	CH bend
C-H of alkane	1369.8		

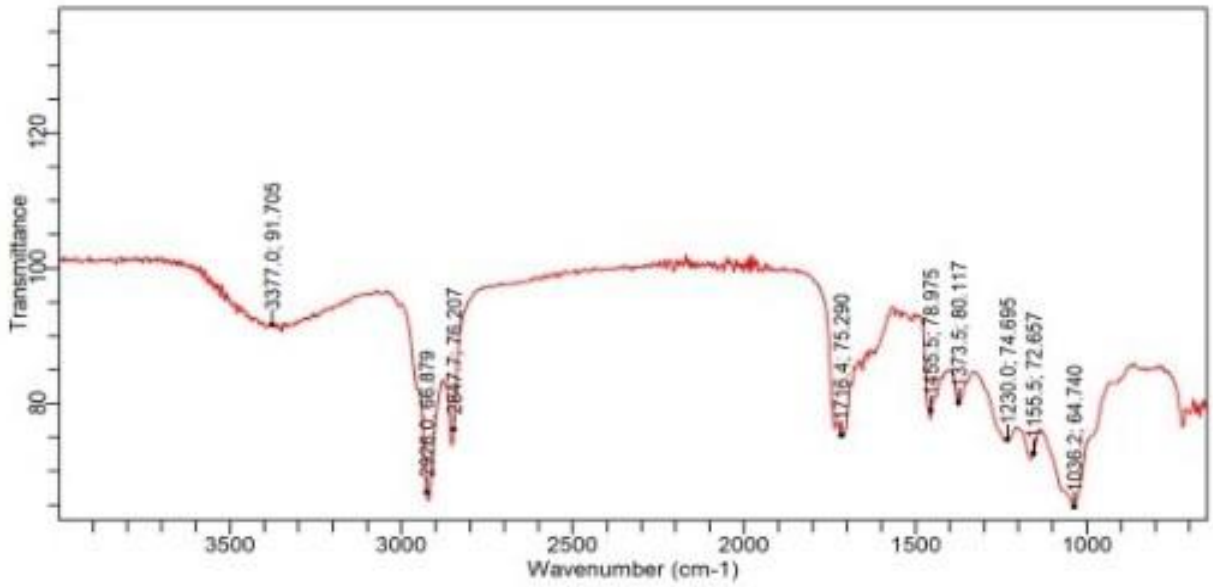

Figure 2: FTIR Analysis of *D. horizontalis* ethyl acetate extract

Table 6: FTIR data of *D. horizontalis* chloroform Extract

Bands	**υ (cm^{-1})**	**Inference**
-OH alcohol	3276.3	O-H intermolecular bonded OH or COOH.
C-H of aldehyde or carbonyl	2914.8	C-H stretching of carbonyl group
C-H of alkane	2847.7	C-H stretching of alkane
C=O of carbonyl	1735.1	C=O stretching of carboxylic acid
C-H of alkane	1459.3	C-H (alkane bending) of methylene group
C-H of alkane	1244.9	C-H (alkane bending) of dimethyl group

Discussion

The results of sequential solvent extractions of the *D. horizontalis* aerial parts (Table 1) showed comparable percentage yields in the solvents used for the extraction. The percentage yield of the extracts showed that ethyl acetate had the highest percentage yield, this variation could be due to the polarity [5]. The plant is more abundant in moderately polar compounds hence ethyl acetate extracts more bioactive polar compounds which are useful in ethno medicine.

The phytochemical evaluation of the extracts from aerial part of *D. horizontalis* were conducted on chloroform and ethyl acetate extract. The findings of this analysis (Table 2 and 3) revealed that the chloroform extract contained Steroids/Triterpenes and Cardiac glycoside. The positive result in steroid, terpenoids and cardiac glycoside support the medicinal use of this plant

and its potential health benefit on microbial infection. The antimicrobial properties, anti-inflammatory effect and anticancer activity of terpenoids suggest that, they possessed active pharmacological potential for the treatment of infections, and inflammation [9]. The presence of steroid in this extract shows that it can be employed in the treatment of asthma, as well as immunosuppression. The ethyl acetate extract detected the presence of flavonoids, steroids/Triterpenes, tannins, alkaloids, cardiac glycoside, phenol, saponins, antraquinones and carbohydrate. The identification of phytochemicals in this work indicates that the extracts of *D. horizontalis* may possess therapeutic properties against pathogens, which could perhaps explain their historic use in treating various disorders [10].

TLC analysis of the extracts revealed the constituent's separation with different Rf values. Tables 4 showed the various R_f values of the distinct spots observed when the extracts of the *D. horizontalis* were chromatographed. The R_f values were found at different spots both under UV light (254 nm and 365 nm) and unaided eye, this suggests that the extracts contain several compounds of different classes [11]. Optical activity of any compound is its ability to rotate the plane of polarized light that passes through it [12].There is a high tendency that the aerial parts of *D. horizontalis* would contain compounds with a specific rotation. However, the scope of this study could not permit in-depth of the information.

Fourier Transform Infrared spectrometer (FT-IR) is the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytoconstituents [13]. The FTIR analysis revealed the presence of various characteristics functional groups in both the chloroform and ethyl acetate extract. The extracts of *D. horizontalis* were found to contain commonly alcohols, aldehydes or carbonyl compounds, alkanes, amines, phenol and nitro compounds among others. These was confirmed from FTIR spectra which revealed the presence –NH, -CH, -CH_3. –C=O, C-C, C=C, -OH, S=O and –NO (Table 5 and 6). This finding aligns with the research conducted by Odo *et al.* (2017), which presented evidence supporting the existence of secondary metabolites and other phytocomponents in plants using FTIR. The absorption of bands at 3373.2 cm^{-1} might be due to bonded O-H stretching vibration of alcohol [14]. The band at 2918.5 cm^{-1} might be due to the presence of C-H of aldehyde or carbonyl compounds. The band at 1712.7 cm^{-1} was attributed to C=O of conjugated aldehyde [7], while alkane presence was indicated by the presence of a band at 2918.5 cm^{-1} [15]. The band (Table 1; 1; 1463.0 cm^{-1}) might be due to the presence of C-H bending of methylene [15]. These compounds have also been reported by previous studies carried out by Odo *et al*., [16] to have absorption of bands at 3000-3880 cm^{-1},

3700-3750 cm^{-1}, and 3200-3600 cm^{-1} due to the bonded O-H stretching vibration of alcohol and absorption of a band at 2700-300 cm^{-1} due to the presence of C-H stretch of alkane. By comparison, this validated the presence of –OH and –CH obtained in the FTIR chart of *D. horizontalis* chloroform extract. The studies' findings highlight the importance of these functional groups in the chemical behavior of *D. horizontalis* extracts. Furthermore, the data suggests a correlation between the identified vibrational bands and the biological activities associated with these compounds.

Conclusion

In the course of the study, the aerial parts (extracts) of *D. horizontalis* (precisely the leaves and the stem bark) were evaluated for chromatographic activity (TLC) and determination of chemical functional groups.

TLC profiles of the extracts of *D. horizontalis* showed the possibility its optical activities due to visual response to UV lamp at designated wavelengths. There are possibilities that the compounds from the aerial parts (leaves and stem bark) of *D. horizontalis* may be optically active. Confirmation of particular functional groups from the examined FTIR spectra suggests the possibility of chemical compounds in *D. horizontalis* leaves and stem bark. Whether the compounds are bioactive or not could not be determined due to the limited scope of the study.

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