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Biochemical, Fatty Acid, Antimicrobial and FTIR Analysis of Flower Petals and Leaves of Different Medicinal Plants.

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ABSTRACT

The Biochemical characterization, Fatty Acid, FTIR and Antimicrobial analysis for five selected medicinally important plants (*Millingtonia hortensis, Bauhinia purpurea, Couroupita guianensis, Cassia fistula* and *Spathodea campanulata*) were performed. The total carbohydrate ,protein, anti microbial and fatty acid analysis was studied. The ethanolic extraction of leaves and flowers of these selected plants were used to perform biochemical and FTIR analysis. The carbohydrate content present in *Millingtonia hortensis* (Leaf:1.569 (mg/ml) , Petal:3.88 (mg/ml)), *Bauhinia purpurea* (Leaf:2.19 (mg/ml) , petal:5.584 (mg/ml)) , *Couroupita guianensis* (Leaf:4.808 (mg/ml) , Petal:3.456 (mg/ml)) , *Cassia fistula*(Leaf:3.11 (mg/ml), Petal:1.672 (mg/ml)), *Spathodea campanulata* (Leaf:5.038 (mg/ml) , Petal:2.967 (mg/ml)). The protein content for different plants in are *Millingtonia hortensis* (Leaf :54.89 (mg/ml), Petal:70.29 (mg/ml)) , *Bauhinia purpurea* (Leaf:40.59 (mg/ml) , Petal:55.99 (mg/ml)) , *Couroupita guianensis* (Leaf:57.09 (mg/ml)) , Petal:53.79 (mg/ml)) , *Cassia fistula (*Leaf:65.89 (mg/ml) , Petal:50.49(mg/ml)), *Spathodea campanulata* (Leaf:61.49 (mg/ml) , Petal:59.39 (mg/ml)). Fatty acid profiles of some selected plants had shown the presence of some medicinally important free fatty acids like ɣ-Linolenic acid in highest percentage of 13.1% in *Spathodea campanulata* petal extract and highest percentage of α-Linolenic acid is found in *Couroupita guianensis* leaf extract as 20.3 %. Antimicrobial analysis was performed for selected plants among which *Millingtonia hortensis* showed antimicrobial activity against *Bacillus subtilis* with zone of inhibition 0.9cm in ethanol leaf extract ,0.85cm in methanol leaf extract. FTIR analysis reveals the presence of functional groups like C-H bond stretching, C-F Stretching.

Keywords: Antimicrobial, Zone of inhibition , FTIR, ɣ-Linolenic Acid.

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INTRODUCTION

Millingtonia hortensis popularity lies in its ornamental value, leaves of this plant are used as antipyretic, sinusitis, cholagogue and tonic in folklore medicine. Stems and roots of the Cork tree have great medicinal value. Its dried flower is a good lung tonic .It is also used in the cough diseases. Its bark is used to produce yellow dye. *Bauhinia purpurea* isvery popular as ornamental tree in subtropical and tropical climates; it is cultivated for its fragrant flowers. In the Neotropics, can be used to attract hummingbirds as Amazilia lacteal , Chlorostilbon lucidus or white-throated hummingbird-in gardens and parks. *Couroupita guianensis* contains the brazil nut and paradise nut . The flowers are used to scent perfumes and cosmetics. The hard shells of the fruit are sometimes used as containers. Fruits are edible and are occasionally eaten, but the smell of the white flesh discourages most people from trying them . *Cassia fistula* is a popular ornamental plant and is also used in herbal medicine. In Ayurvedic medicine, the golden shower tree is known as aragvadha, meaning "disease killer". *Spathodea campanulata* is planted extensively as an ornamental tree throughout the tropics and is much appreciated for its very showy reddish-orange or crimson, campanulate flowers. In Ghana, the bark and leaves are used in traditional medicine. A brew made from the bark, leaves and flowers of this tree is used for treating various illness. We have done biochemical analysis for those flowers by using Anthrone method and Lowry's method. We have done FTIR analysis for those selected plants. The FTIR involves the application of traditional infrared spectroscopy to low concentration measurements, such as ambient air measurements, is limited by several factors. First is the significant presence of water vapour , CO2 and methane , which strongly absorb in many regions of the infrared(IR) spectrum. Fatty acid is a long hydrocarbon chain which is capped by a carboxyl group. These are usually derived from triglycerides or phospholipids. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP .In our analysis of fatty acid in those five plants we have got tricosanic acid as highest content as 30.2% in *Millingtonia hortensis* leaf extract and Heptadecanoic acid methyl ester as 21.5% in *Spathodea campanulata*.Antimicrobial activity is anything that destroys bacteria or suppresses their growth or their ability to reproduce. Heat , chemicals such as chlorine, and antibiotic drugs all have antibacterial properties. In our analysis, *Millingtonia hortensis* showed antimicrobial activity against bacillus subtilis.

EXPERIMENTAL

Materials

All the chemicals required for experiment were persuaded from Merck and high grade HPLC solvents were used.

Methods

Estimation of Proteins by Lowry's Method

We had pipetted out 0.2-1ml of the protein solution into a series of test tubes and volume was made to 4ml by adding water. The blank tube contained only 1ml of water. To each tube , 5ml of the alkaline copper solution was pipetted out , and it was mixed well and allowed to stand at room temperature for 10 min. 0.5ml of FC reagent was pipetted out into each tube by mixing rapidly after each addition . We had kept the test tubes at room temperature for 30 min. The colour formed was measured at 650nm. A proper blank without protein had been used. Standard graph was drawn with the concentration on X-axis and OD on Y-axis. The same procedure was followed to know the OD of unknown sample and we had interpolated its value using standard graph.

Estimation of Carbohydrates by Anthrone Method

We had pipetted out different volumes of glucose solution ranging from 20-100µg into a series of test tubes. The volume was made to 1mL by adding water. To each tube we had added 4mL of Anthrone reagent , mixed well and covered the tubes with marbles on top and kept them in a boiling water for 10 minutes. We had cooled the test tubes to room temperature and measured the OD at 620nm using a blank tube containing 1mL water. Standard graph was drawn and Beer's law was verified. We had carried out similar procedure for estimation of unknown glucose solution. Corresponding OD values were taken and concentration was calculated from the graph.

Fatty Acid Analysis by Gas Chromatography

The leaves and flowers of different plants were collected and they were sundried till the moisture was removed and they were crushed into fine powder. 20 ml of freshly prepared trans esterification reagent (methanol/acetyl chloride, 95:5 v/v) was added to 100mg of dried biomass powder of leaves and petals in a crew capped bottle and it was heated at 80°C for 1 hour. The mixture was cooled to room temperature and 2-3 mL of water was added into each tube. Methylated Free Fatty Acids were extracted by adding hexane (twice the volume of the reagent) for phase separation. Hexane phase was separated and evaporated to 1mL for GC analysis.

FTIR Analysis

Ethanol extracts were prepared for leaves and petals of all collected samples and were applied directly to the potassium bromide (KBr) glass windows with 4µ path length. All the spectra were smoothed and analyzed by using Spectrum 10 version 10.3.06 software.

RESULTS AND DISCUSSION

Analysis of Carbohydrate Content

The carbohydrate content present in leaves and petals of different plants was estimated by using Anthrone Method and the results were shown in Fig 1. The highest amount of carbohydrate was observed in petals of *Bauhinia purpurea* which is found to be 5.584(mg/ml) and least amount of carbohydrate content was observed in leaves of *Millingtonia hortensis* which is found to be 1.569(mg/ml).

Analysis of Protein Content

The protein content present in leaves and petals of different plants was estimated by using Lowry's Method and the results were tabulated below in Fig 2.The highest amount of protein was observed in petals of *Millingtonia hortensis* which is found to be 70.29(mg/ml) and least amount of protein content was observed in petals of *Cassia fistula* which is found to be 50.49(mg/ml).

Carbohydrates and Proteins are an important part of diet across the world which can increase the metabolism in our body by consumption. The present study is performed in order to know the amount of Protein and Carbohydrate content present in leaves and petals of different flowers. As the leaves of these are plants are consumable they can used as protein and carbohydrate supplements to the body. Malnutrition[1] is a commonly seen disease and a high protein source diet with other supplements of vitamins and minerals can cure it. Deficiency of proteins in our body can lead to diseases such as Marasmus and kwashiorkor and only a complete balanced diet can help us to get rid of these diseases .[2]

FTIR Analysis

FTIR spectrum of different plants with Ethanol as solvent has showed prominent peaks which are showed from Fig3 to Fig12 . Table 1 describes prominent bond stretching observed in FTIR Analysis .By analyzing the results we can observe that almost all the frequencies lie in between the range of 2800-3000 cm^{-1} shows prominent peak C-H Bond Stretching [3] and other prominent peak at the frequency 1386.7 cm^{-1} is C-F Stretching [4]

Fatty Acid Analysis

Fatty Acid is a long hydrocarbon chain capped by a Carboxyl group . The Fatty Acid Analysis of these selected plants reveals the presence of various Free Fatty Acids among which Linolenic Acids which are of medicinal importance were observed in highest percentage ɤ-Linolenic Acid of 13.1% in *Spathodea campanulata* petal extract and highest percentage of α-Linolenic Acid is found in *Couroupita guianensis* leaf extract as 20.3%. The Fatty Acid profiles of leaves and petal extracts in different plants is described below in the table 2.There are also certain Essential Amino Acids like Linolenic acid (omega 6 fatty acid) and it has numerous functions on human body like formation of healthy cell membranes,[5] Proper development and

functioning of the brain and nervous system, Regulation of blood pressure, liver function, immune and inflammatory responses, Regulation of blood clotting, Crucial for the transport and breakdown of cholesterol.[6]

Antimicrobial Activity

The results of Antimicrobial activity of leaves and petals in different plants against solvents like Ethanol and Methanol were tabulated below in Table 3 . An Antimicrobial agent is an agent that kills microorganisms or inhibits their growth[7]. Ethanolic and Methanolic extracts were used for studying antimicrobial activity against 3 pathogenic species Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae. The antimicrobial activity has been observed in *Millingtonia hortensis* against Bacillus subtilis and high susceptibility is recorded high in case of Diluted Ethanol Leaf Extract[8]. Plants consist of other substances such as secondary metabolites which are not involved directly in normal growth , development, reproduction of organism but they are important in plant defence against plant herbivory and other interspecies defenses such as alkaloids, flavonoids, glycosides, terpenoids. [9,10]

Table 1: The Functional Groups corresponding to the particular frequency of the sample plants have been listed below in the table

Sample	Frequency(cm-1)	Functional Group
Millingtonia hortensis Leaf	2930.8	C-H Bond Stretching
	2896.8	C-H Bond Stretching
	2855.1	C-H Bond Stretching
	1386.7	C-F Stretching
Millingtonia hortensis Petal	2904.3	C-H Bond Stretching
Bauhinia purpurea Leaf	2896.8	C-H Bond Stretching
Bauhinia purpurea Petal	2904.3	C-H Bond Stretching
Couroupita guianensis Leaf	1383	C-F Stretching
Couroupita guianensis Petal	2927	C-H Bond Stretching
	2896.8	C-H Bond Stretching
Cassia fistula Leaf	2904.3	C-H Bond Stretching
	2983.8	C-H Bond Stretching
	1386.7	C-F Stretching
Cassia fistula Petal	2923.3	C-H Bond Stretching
	2983.8	C-H Bond Stretching
	1386.7	C-F Stretching
Spathodea campanulata Leaf	2919.5	C-H Bond Stretching
	1386.7	C-F Stretching
Spathodea campanulata Petal	2904.3	C-H Bond Stretching

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Table 2: The Fatty Acid composition of leaves and petal extracts in different plants is described below in the table

A-*Millingtonia hortensis* leaf extract
B-*Millingtonia hortensis* petal extract

FATTY ACID	A	B	C	D	E	F	G	H	I	J
CapricAcid (10:00)	11.3	4.3	21.1	3.2	-	-	5.1	-	8.6	20.5
HeptadecanoicAcid (17:00)	9.8	-	13.6	9.6	3.1	-	5.7	7.5	9.4	-
ɤ-Linolenic Acid(18:3n6)	9.2	5.8	10.2	8.4	8.4	0.5	8	8.4	9.8	21.5
Linolenic Acid (18:3n3)	9.2	9.8	7.8	-	20.3	1.4	20.2	-	7.4	13.1
Tricosanic Acid(23:0)	30.2	5.9	-	-	-	-	-	-	-	13.8
Undecanoic Acid(11:00)	-	-	-	0.9	3.9	-	-	12.2	-	-
Cis-11,11 EicosadienoicAcid (15:01)	-	1.1	13.4	-	-	0.6	-	11	19	-
Palmitic Acid (16:00)	-	-	-	-	-	3.3	-	3.5	-	-
Cis-10-pentadecanoicAcid (15:01)	-	-	-	-	-	-	0.1	-	-	-

C-*Bauhinia purpurea* leaf extract
D-*Bauhinia purpurea* petal extract
E-*Couroupita guianensis* leaf extract
F-*Couroupita guianensis* petal extract
G-*Cassia fistula* leaf extract
H-*Cassia fistula* petal extract
I- *Spathodea campanulata* leaf extract
J-*Spathodea campanulata* petal extract

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Table 3:The results of Antimicrobial activity of leaves and petals in different plants against solvents like Ethanol and Methanol were tabulated below

Extract	distance from centre(cm)	Zone of Inhibition(cm)
ethanol leaf extract	0.35	0.9
methanol leaf extract	0.45	0.85

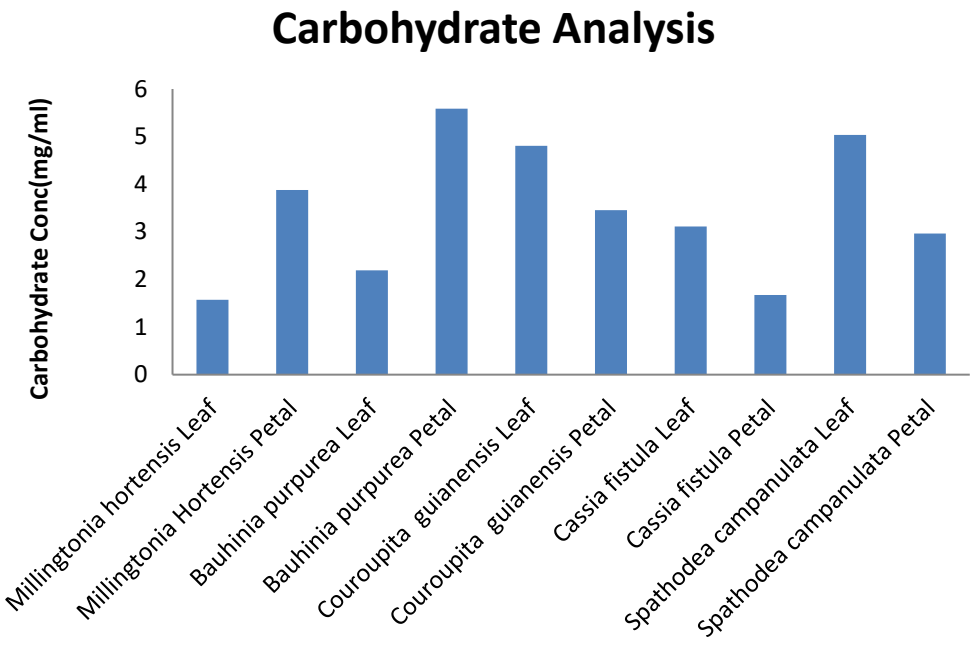

Fig.1 Carbohydrate concentration of different selected medicinal plants

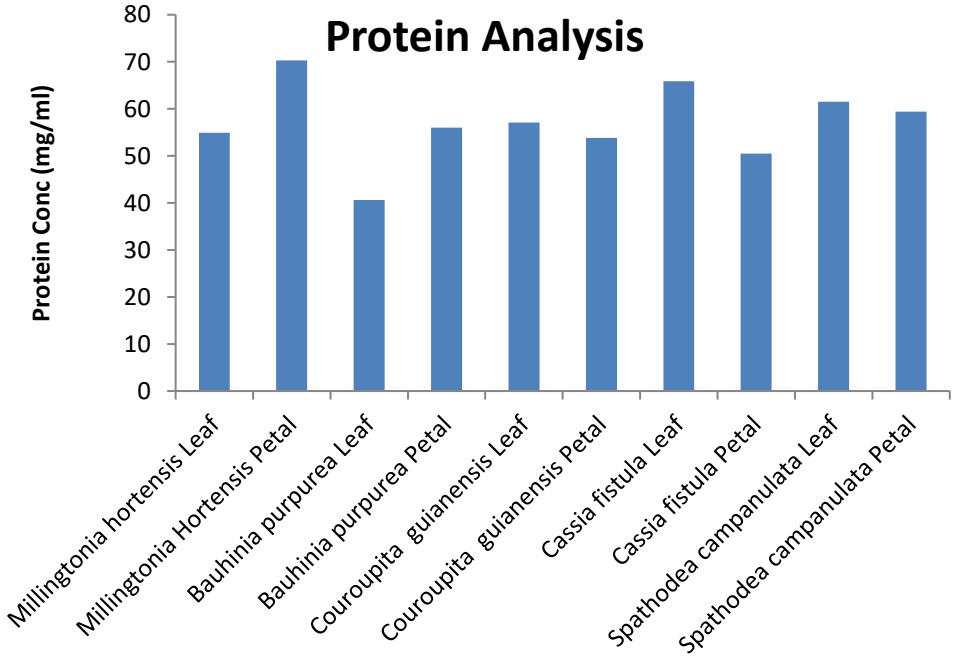

Fig. 2: Protein Concentration of different selected medicinal plants

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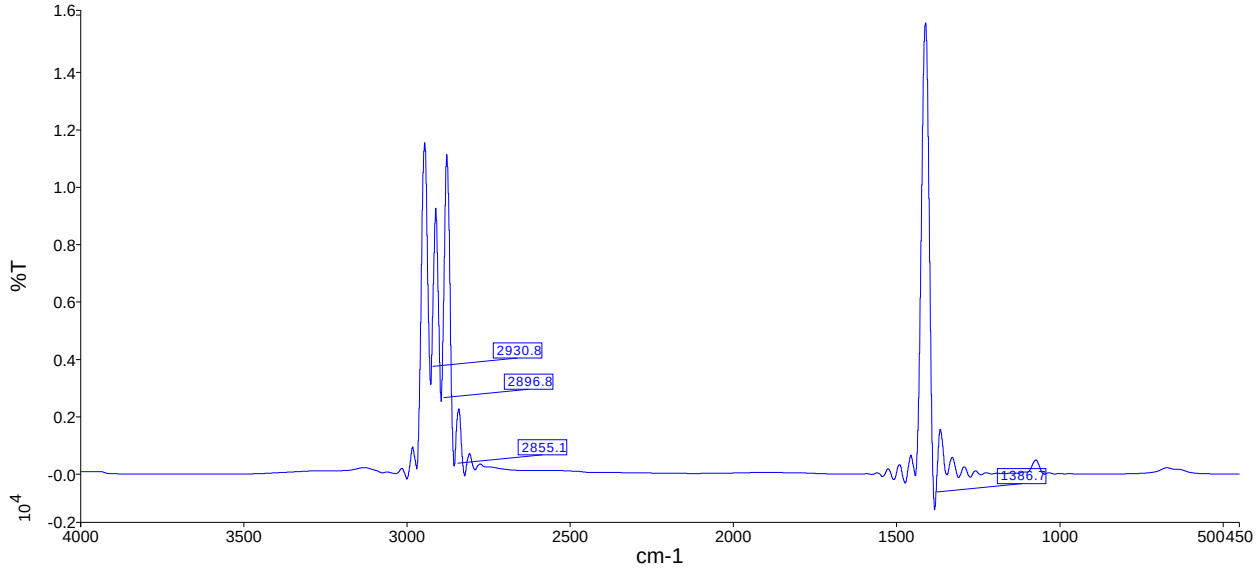

Fig. 3: Image of *Millingtonia hortensis* Leaf Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2930.8,2896.8,2855.1 and the functional GroupC-F Bond Stretching at frequency of 1386.7

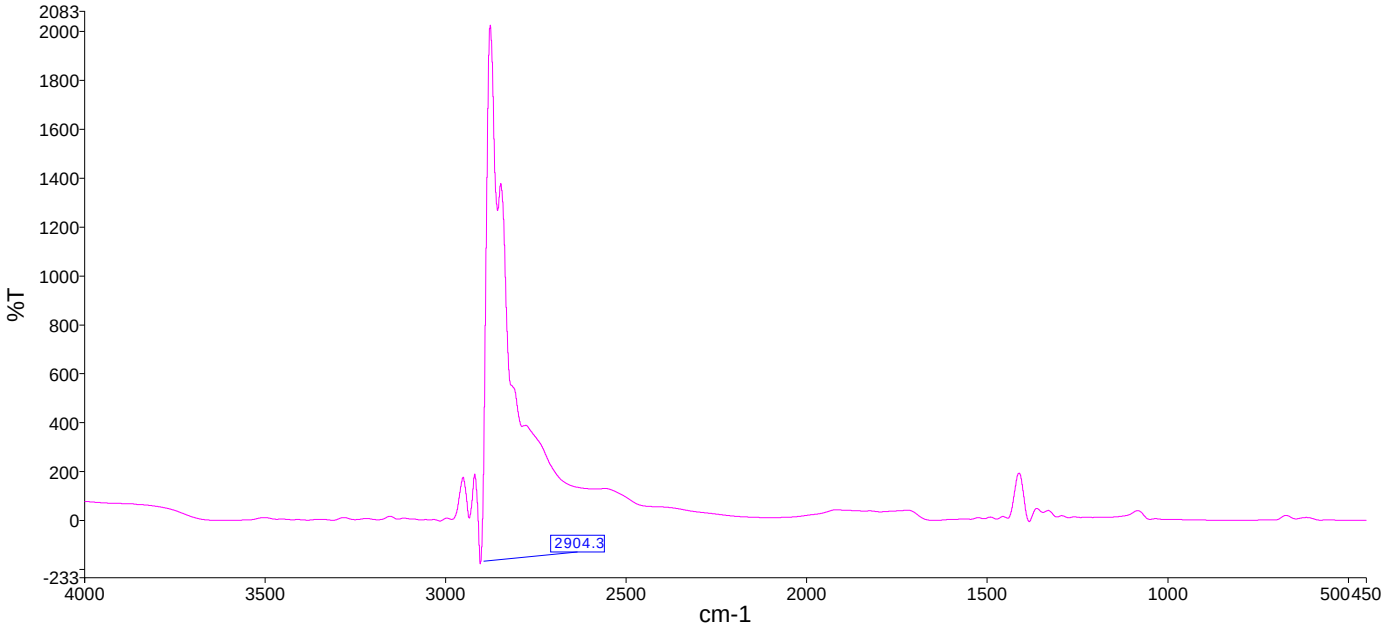

Fig. 4:Image of *Millingtonia hortensis* Petal Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2904.3

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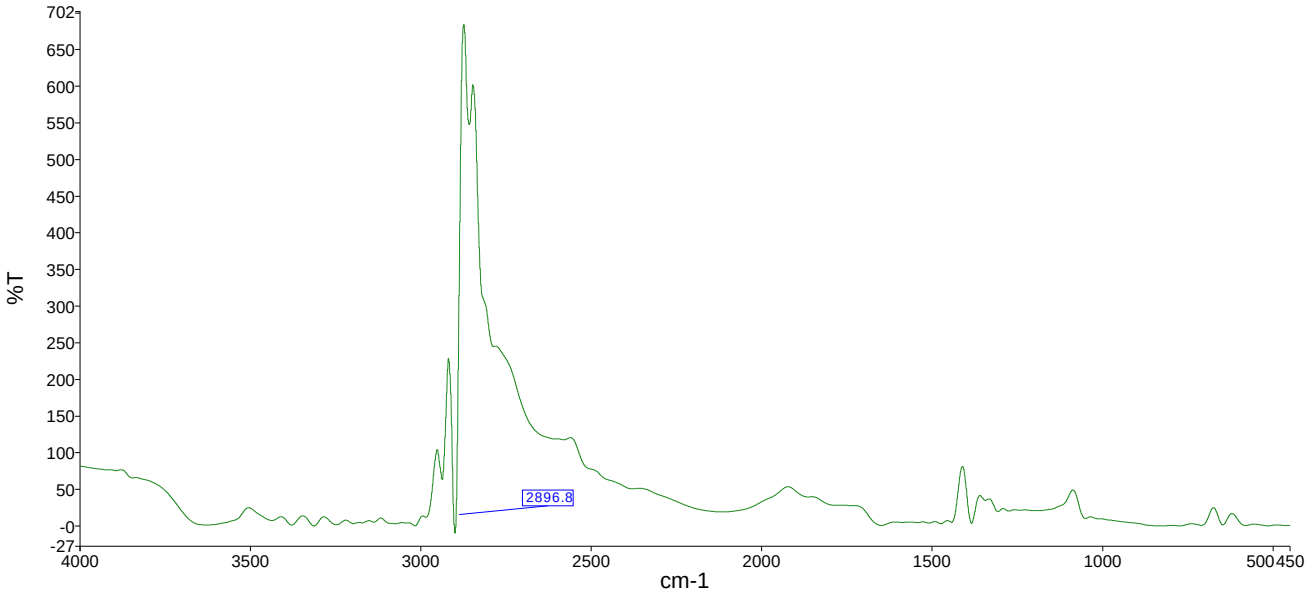

Fig. 5:Image of *Bauhinia purpurea*Leaf Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2896.8

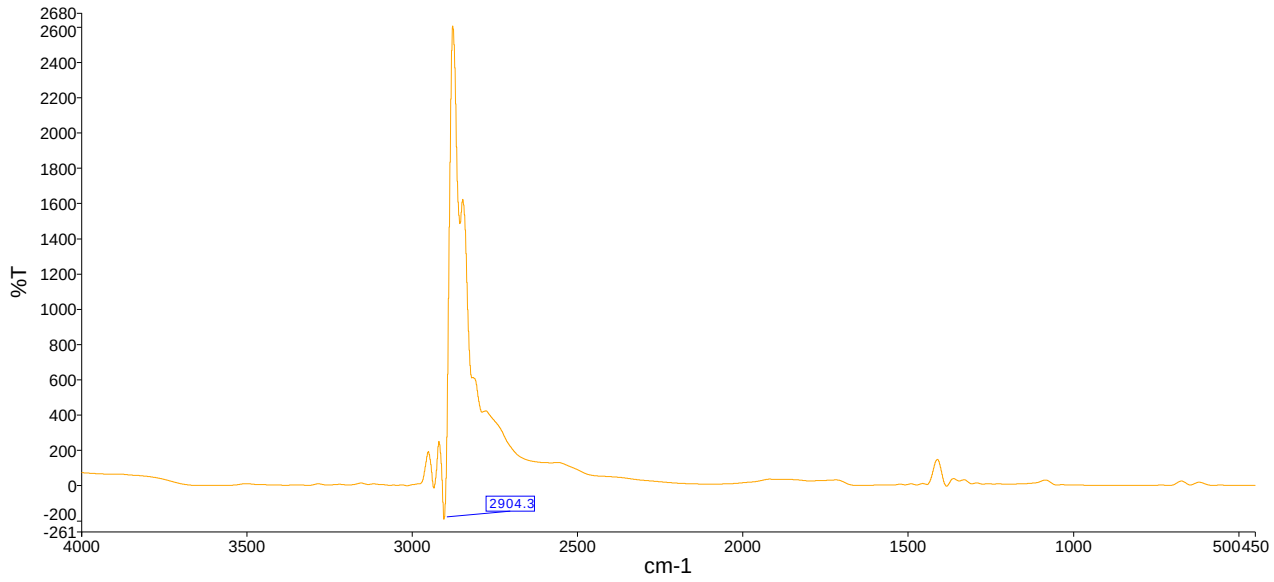

Fig. 6:Image of *Bauhinia variegate* Petal Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2904.3

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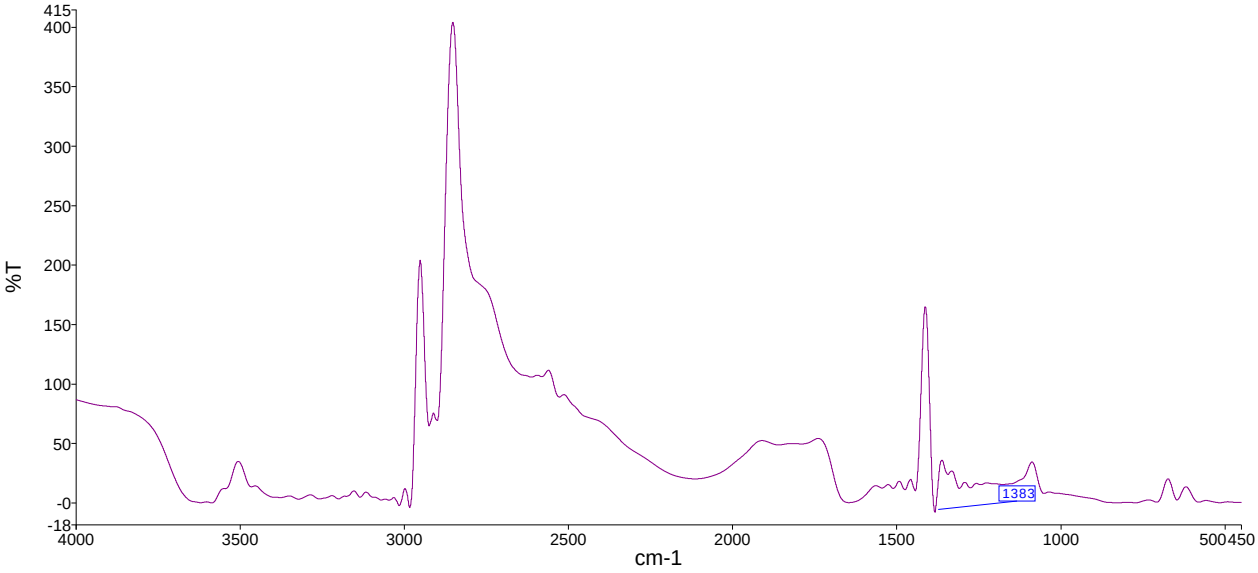

Fig. 7:Image of *Couruptia guianensis* Leaf Ethanol Extract with the functional GroupC-F Stretching at frequency of 1383

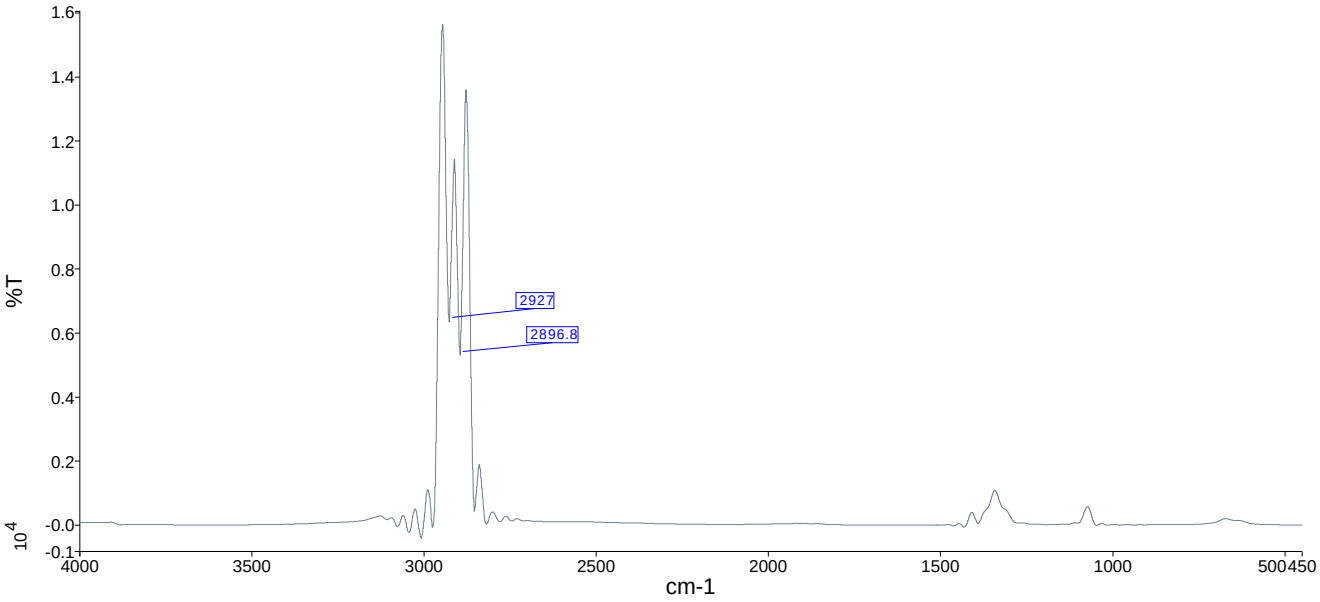

Fig. 8:Image of *Couruptia guianensis* Petal Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2927 and 2986.8

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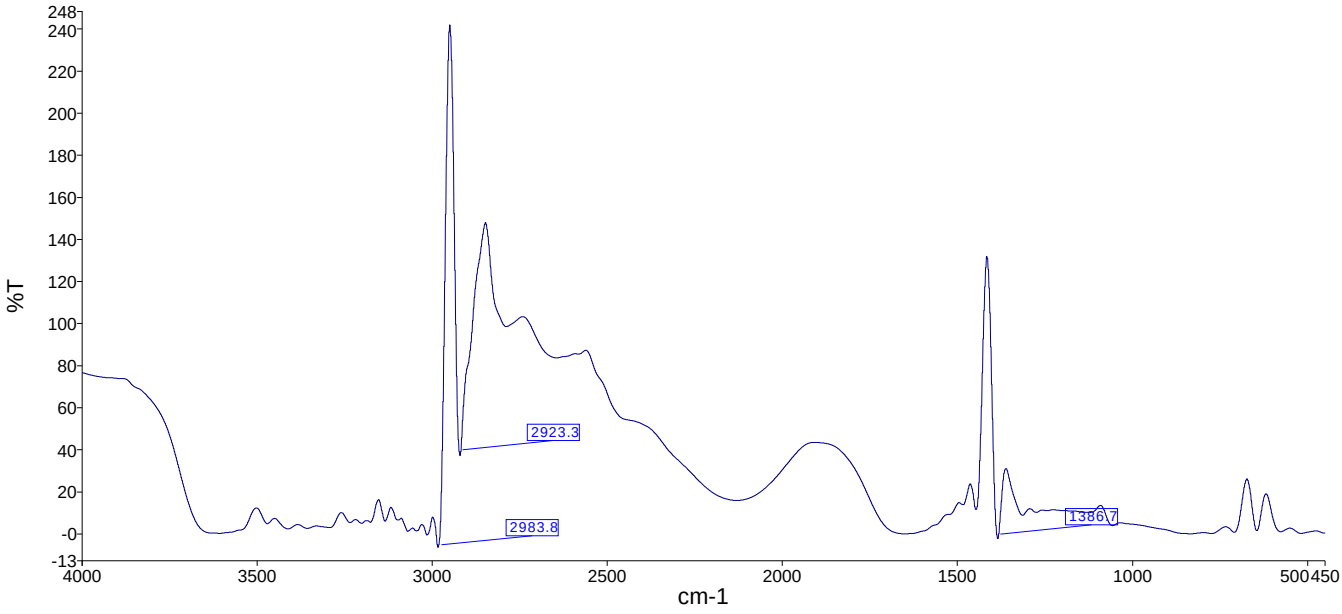

Fig. 9:Image of *Cassia fistula* Leaf Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2904.3,2983.8 and functional GroupC-F Bond Stretching at frequency of1386.7

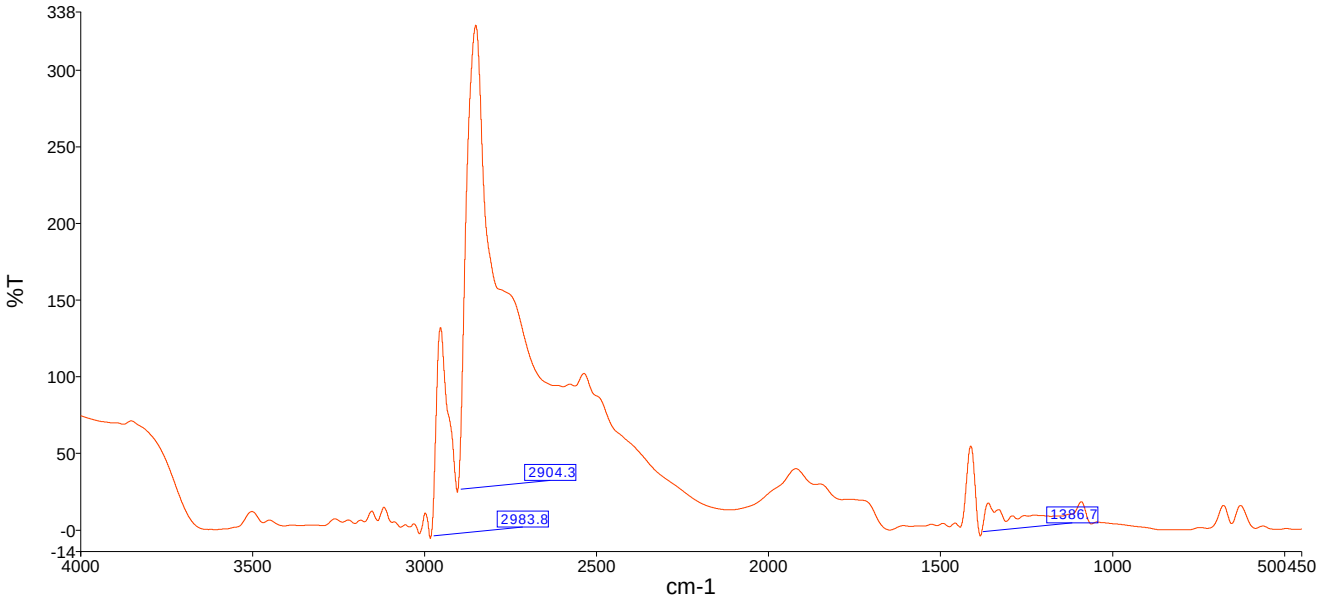

Fig. 10:Image of *Cassia fistula* Petal Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2923.3 2983.8 and the functional group C-F stretching at frequency of 1386.7

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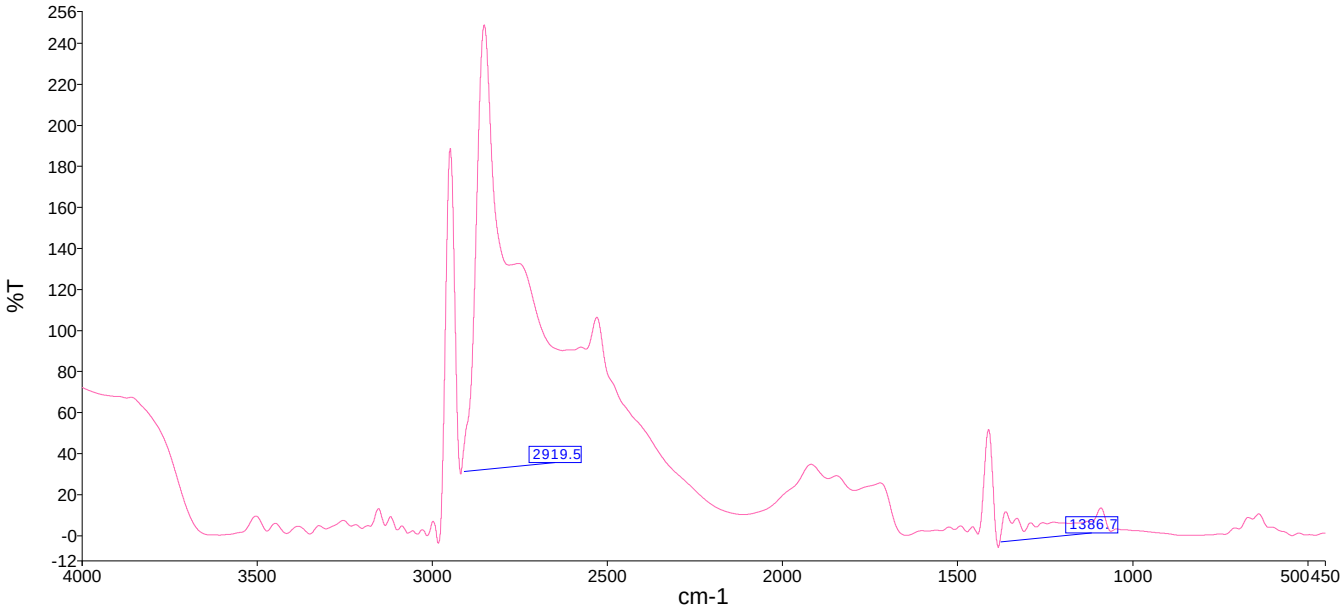

Fig. 11:Image of *Spathodea campanulata* Leaf Ethanol Extract with the functional GroupC-H Bond Stretching and C-F stretching at frequency of 2919.5 and 1386.7

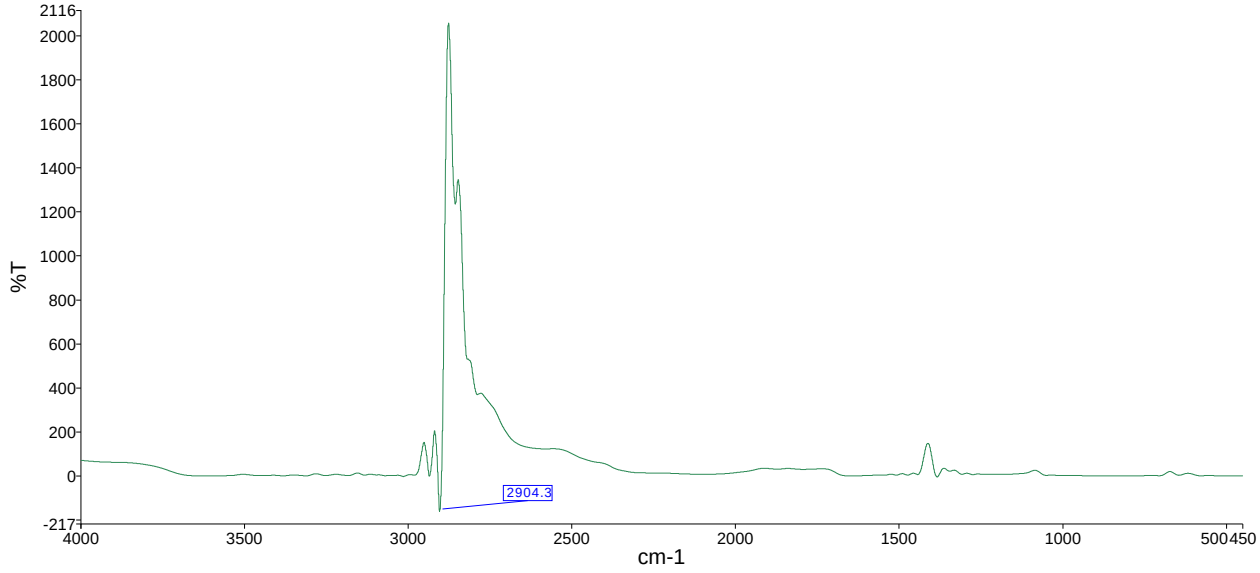

Fig. 12:Image of *Spathodea campanulata* Petal Ethanol Extract with the functional GroupC-H Bond Stretching at frequency of 2904.3

CONCLUSION

The carbohydrate content present in *Millingtonia hortensis* (Leaf:1.569(mg/ml), Petal:3.88(mg/ml)), *Bauhinia purpurea* (Leaf:2.19 (mg/ml) ,Petal:5.584 (mg/ml)) ,*Couroupita guianensis*(Leaf:4.808 (mg/ml) , Petal:3.456 (mg/ml)) , *Cassia fistula*(Leaf:3.11 (mg/ml), Petal:1.672 (mg/ml)) , *Spathodea campanulata* (Leaf:5.038 (mg/ml) , Petal:2.967 (mg/ml)). The protein content for different plants in are *Millingtonia hortensis* (Leaf :54.89 (mg/ml) , Petal:70.29 (mg/ml)) , *Bauhinia purpurea* (Leaf:40.59 (mg/ml) , Petal:55.99 (mg/ml)) , *Couroupita guianensis* (Leaf:57.09 (mg/ml)) , Petal:53.79 (mg/ml)) , *Cassia fistula (*Leaf:65.89 (mg/ml) , Petal:50.49 (mg/ml)) , *Spathodea campanulata* (Leaf:61.49 (mg/ml) ,Petal:59.39 (mg/ml)). The Fatty acid analysis has shown the presence of some medicinally important free fatty acids such as linolenic acid and tricosanic acid. FTIR analysis reveals the presence of functional groups like C-H stretching and C-F bond

stretching. The ethanolic and methanolic extracts of Millingtonia hortensis had shown zone of inhibition which can further be analysed and used as antimicrobial agents.

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