FT- IR ANALYSIS FOR THE DETERMINATION OF FUNCTIONAL GROUPS PRESENT IN SOME ORGANIC COMPOUNDS FROM THE WHOLE PLANT OF *Chromolaena odorata* L.*

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ABSTRACT

In this research, the whole plant of *Chromolaena odorata* L. was selected for the chemical analysis. Phytochemical screening was done by means of standard method. Antimicrobial activities of the crude extracts of the sample were tested by Agar-well diffusion method on six selected organisms. The antioxidant activity of ethanol extract of the whole plant of *Chromolaena odorata* L. was determined by using DPPH assay. The elemental compositions of the crude extract were determined by EDXRF spectral data. Compounds (1, 2, 3 and 4) were isolated from the whole plant of *Chromolaena odorata* L. using Chromatographic separation method. Finally, the functional groups of the isolated compounds were determined by FT-IR spectral data.

Keywords : *Chromolaena odorata* L.; EDXRF spectral data; DPPH assay; FT-IR spectroscopy

1 INTRODUCTION

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people [1]. They serve a therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines. Parts of such plant, including leaves, stems, barks, flowers, roots, rhizomes, fruits, grains or seeds are used for controlling or treating a disease condition and as such may be the sources of medically or biologically active chemical substances [2]. Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine. Many food crops have medicinal effects, for example garlic. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species.

Chromolaena odorata L. is an herbaceous perennial that grows to a height of three meters in open situation and up to eight meters when assumed a scrambling habitat in the interior forests [3]. It has the reputation of using as a medicinal herb for a variety of ailments including malaria, fever, and the aqueous leaf extract of the plant is used as antiseptic. The fresh leaves and extract of *C. odorata* L. are used in traditional herbal treatment in developing countries for burns, soft tissue wounds and skin infections [4]. In folk medicine, a decoction of the leaf is used as a cough remedy. The literature reveals that the leaves of *C. odorata* (L). are also used against sexually transmitted diseases [5]. Furthermore, it has also been shown to possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant properties [6].

2 MATERIALS AND METHODS

2.1 Materials

Commercial grade reagents and solvents were used without further purification. The whole plant of *Chromolaena odorata* L. was collected from Patheingyi Township, Mandalay Region, Myanmar. UV-VIS Spectrometer (Shimadzu, Japan) was used for the determination

of antioxidant activity. EDXRF spectrophotometer (AMETEX, England) was applied to determine the chemical elements in the sample. Silica gel (Merck Co. Inc Kiesel gel 60 F_{254}, 70-230 mesh) was used for Column Chromatography. ***UV-Lamp (L***ambda – 40, Perkin – Elmer Co, England) and iodine vapor were used as developing agents in column chromatography. FT-IR spectrometer (Shimadsu, Japan) was used for the identification of the functional groups of the isolated compounds.

2.2 Preliminary Phytochemical Screening

The phytochemical tests were carried out at Department of Chemistry, University of Mandalay, Myanmar to detect the different kinds of chemical constituents in the sample [7] [8] [9] [10].

2.3 Determination of Antimicrobial Activity on Crude Extracts

The antimicrobial activities of the crude extracts from the whole plant of C*hromolaena odorata* L. were tested by Agar-well diffusion method on six selected microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli* in PRD (Pharmaceutical Research Department), in Yangon, Myanmar [11].

2.4 Determination of Antioxidant Activity by DPPH Assay

In this experiment, 1, 1-diphenyl-2-picryl hydrazyl (DPPH) powder was used as stable free radical according to Manzocco and *et al*., 1998. (Manzocco, 1998) Ascorbic acid was used as standard antioxidant. Ethanol (Analar grade) was also used as solvent. The absorbance was determined at 517 nm wavelength by using UV- VIS spectrophotometer [12] [13].

2.5 Determination of Elemental Compositions in the Sample by EDXRF Method

The elemental compositions in the whole plant of *Chromolaena odorata* L. were determined qualitatively and quantitatively by using EDXRF at Department of Chemistry, University of Monywa, Myanmar.

2.6 Extraction and Isolation of Pure Organic Compounds

The air dried sample (500g) was percolated with 95% ethanol (3L) at room temperature for about two months. The ethanol crude extract was again extracted with ethyl acetate (250 mL) under normal condition. The ethyl acetate crude extract (4 g) was packed with 50 g of silica gel in a column (55cm x 2.5 cm) and eluted with n-hexane; n-hexane: ethyl acetate mixtures (19: 1- 1: 19) and ethyl acetate. The progress of separation was monitored by thin layer chromatography using n- hexane: ethyl acetate mixtures. Ten combined fractions with same R_f values were obtained. The combined fractions IV, VI, VIII and IX have found to be main portions. From the microcolumn separation with same solvent systems, combined fraction IV, VI, VII and IX gave the compounds (1-4) as white crystal form. The weights of the pure compounds were found as 11.2 mg, 16.1 mg, 9.5 mg and 19.5 mg respectively. The yield percentages could be calculated as 0.28%, 0.40%, 0.24% and 0.48% based upon the crude ethyl acetate extract [14].

Fig 1. Column chromatographic separation of the sample

3 RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Tests of the Whole Plant of *Chromolaena odorata* L.

The results obtained for the phytochemical screening from the whole plant of C*hromolaena odorata* L. showed the presence of alkaloids, phenolic compounds, glycosides, reducing sugars, tannins, saponins, polyphenols, steroids and flavonoids respectively as shown in the table (1).

Table (1) Results of Phytochemical Test of *Chromolaena odorata* L.

No.	Constituents	Reagents	Observation	Results
1.	Alkaloids	(1)Dragendorff's reagent (2)Wagner's reagent	Orange ppt Brown ppt	+ +
2.	Phenolic Compounds	10% $FeCl_3$	Brown color solution	+
3.	Glycosides	10% Lead acetate	White ppt	+
4.	Reducing Sugars	Benedict's solution	Brick red PPt	+
5.	Tannins	10% $FeCl_3$, Conc. H_2SO_4	Yellowish brown ppt	+
6.	Saponins	Shake vigorously	Froth	+
7.	Terpenes	Acetic anhydride, $CHCl_3$, Conc. H_2SO_4	Pink color solution	_
8.	Polyphenols	1%$FeCl_3$,1% K_3 [Fe $(CN)_6$]	Greenish blue color solution	+
9.	Steroids	Acetic anhydride, Conc.H_2SO_4, $CHCl_3$	Greenish color solution	+
10.	Flavonoids	Conc. HCl, Mg turnings	Green color solution	_

(+) = presence of constituents, (-) = absence of constituent

3.2 Antimicrobial Activities of Crude Extracts from the Whole Plant of *Chromolaena odorata* L.

The results of the antimicrobial activities of the crude extract of the sample are given in the table (2).

Table (2) Antimicrobial Activities of Crude Extracts from the Whole Plant of *Chrmolaena odorata* L.

Crude Extract	Diameter of Inhibition Zone (mm)					
Solvent	I	II	III	IV	V	VI
n- hexane	12 (+)	12 (+)	12 (+)	12 (+)	13 (+)	13 (+)
EtOAc	13(+)	13 (+)	13 (+)	14 (+)	14 (+)	14 (+)
EtOH	14 (+)	15 (++)	13 (+)	14 (+)	15 (++)	12 (+)

Microorganisms
I = *Bacillus subtilis* II = *Staphylococcus aureus* III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus* V = *Candida albicans* VI = *E. coli*

Agar well- 10 mm, 10 mm ~ 14 mm (+); 15 mm ~ 19 mm (++); 20 mm above (+++)

According to this table, n-hexane and ethyl acetate extracts of the sample show low activities on all organisms. Ethanol extract showed medium activities on *Staphylococcus aureus* and *Candida albicans* and low activities on activities on *Bacillus sublilis*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *E. coli.*

3.4 Determination of Antioxidant Activity of Ethanol Extract of the Sample by DPPH Assay

The antioxidant activity of the ethanol extract of the whole plant of *Chromolaena odorata* L. was determined in DPPH free radical scavenging assay. From this assay, IC_{50} value of the whole plant of *Chromolaena odorata* L. was found to be 381.003 µg/ml and showed low activity than standard ascorbic acid compared with its IC_{50} (15.518 µg/ml) value. The antioxidant activity results of the ethanol extract from the whole plants using DPPH assay were shown in the table (3).

Table (3) Percent Inhibition of Various Concentrations of Sample

Concentration (µg/mL)	Mean Absorbance	Mean % Inhibition	IC_{50}(µg/mL)
100	0.409	20.428	
50	0.411	20.039	
25	0.434	15.564	381.003
12.5	0.452	12.062	
6.25	0.456	11.284	

IC_{50} value of the ethanol extract of the sample was calculated by using linear regressive equation.

3.5 EDXRF Analysis for the Determination of Elemental Compositions in the Sample

The results of the elemental compositions in the sample by applying EDXRF spectrometer are tabulated in the table (4). According to EDXRF report, there are thirteen elements in the sample and no heavy toxic metal. In addition, the sample contains the highest amount of calcium followed by chloride and potassium.

Table (4.4) Relative Abundance of the Mineral Elements Present in the Whole Plant of *Chromolaena odorata* L.

No	Elements	Symbols	Relative Abundance
1	Calcium	Ca	1.599 %
2	Chloride	Cl	1.456 %
3	Potassium	K	1.211%
4	Magnesium	Mg	0.796 %
5	Silicon	Si	0.739 %
6	Sulfur	S	0.446 %
7	Phosphorus	P	0.220 %
8	Iron	Fe	0.035 %
9	Manganese	Mn	0.006 %
10	Zinc	Zn	0.004 %
11	Titanium	Ti	0.004 %
12	Copper	Cu	0.003 %
13	Strontium	Sr	0.002 %

3.6 Functional Group Determination of Isolated Organic Compounds (1- 4)

The functional groups determinations of the isolated compounds (1, 2, 3 and 4) were done by using the FT-IR spectral data of these compounds [15]. (Silverstein, 1981)

3.6.1 FT-IR Assignment of Isolated Compound (1)

In the FT-IR spectrum of compound (1), the bands at 3422.94 and 3296.41 cm^{-1} indicate the O-H stretching vibration of hydroxyl group. The peak at 2913.90 and 2849.17 cm^{-1} are due to the asymmetric and symmetric C-H stretching vibrations of sp^3 hydrocarbons. The bands which occur at 1460.37 cm^{-1} should be the C-H in plane bending vibration of sp^3 hydrocarbons. The OH in plane bending vibration of alcohol was observed at 1377.99 cm^{-1} The C-O stretching vibration of alcohol group was observed at 1163.19, 1024.90 cm^{-1}. The peak at 995.48 cm^{-1} is due to the C-H out of plane bending vibration of sp^3 hydrocarbons. Finally, the bands at 783.68 cm^{-1} and 727.72 cm^{-1} represent O-H out of plane bending vibration of hydroxyl group. According to the IR spectrum in Figure (2), the compound (1) contains the alcohol and sp^3 hydrocarbon functional groups.

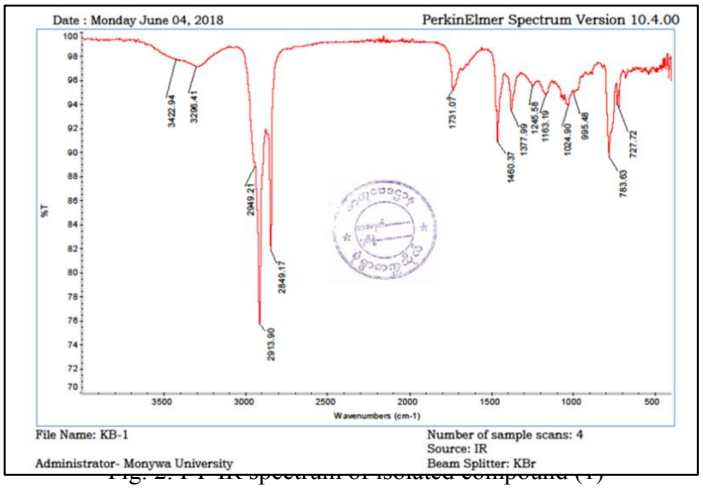

Fig. 2. FT-IR spectrum of isolated compound (1)

3.6.2 FT-IR Assignment of Isolated Compound (2)

In the FT-IR spectrum of compound (2), the band at 3355.36 cm^{-1} represents the O-H stretching vibration of alcohol group. The peaks at 2919.71 cm^{-1} and 2846.12 cm^{-1} indicates the asymmetric and symmetric C-H stretching vibrations of sp^3 hydrocarbons. The peaks at 1462.63 cm^{-1} informed the C-H in plane bending vibration of sp^3 hydrocarbons. The peak at 1380.21 cm^{-1} is due to the O-H in plane bending vibration of alcohol group. Moreover, the peak at 1032.87 cm^{-1} represents C-O stretching vibration of alcohol group. The C-H out of plane bending vibration of sp^3 hydrocarbons is appeared at 994.60 cm^{-1}. Finally, the peak at 782.67 cm^{-1} is due to the O-H out of plane bending vibration of alcohol group. According to these FT- IR spectral data, the compound (2) shows the presence of –OH functional group and sp^3 hydrocarbons.

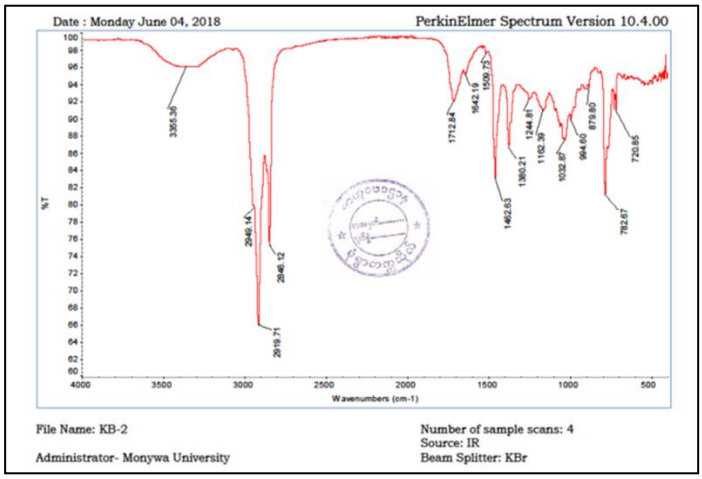

Fig. 3. FT-IR spectrum of isolated compound (2)

3.6.3 FT-IR Assignment of Isolated Compound (3)

The bands at 3428.95 cm^{-1} and 3311.20 cm^{-1} indicates the O-H stretching vibration of alcohol groups. The peak at 3028.62 cm^{-1} assigned the C-H stretching vibration of sp^2 hydrocarbon. The peaks at 2955.03 cm^{-1} and 2916.76 cm^{-1} should be asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbons. The bands which occur at 1462.63 cm^{-1} and 1383.16 cm^{-1} representing the C-H bending vibration of methyl groups. The peak at 965.17 cm^{-1} observed the C-H out of plane bending vibration of E- or trans- alkene group. Finally the and at 835.65 cm^{-1} represent the $=CH_2$ wagging vibration of exo-methylene group.

According to the IR spectrum in Figure (4), the compound (3) shows the presence of –OH functional groups, sp^2 hydrocarbons, sp^3 hydrocarbons, ether and Z- or cis- alkene groups.

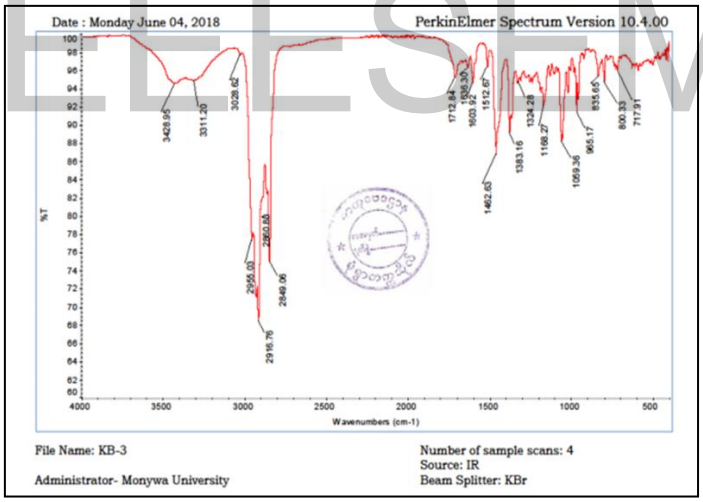

Fig. 4. FT-IR spectrum of isolated compound (3)

3.6.4 FT-IR Assignment of Isolated Compound (4)

According to FT-IR spectrum of compound (4) shown in Figure (5), the bands at 3426.00 cm^{-1} and 3296.48 cm^{-1} were due to the O-H stretching vibration of alcohol groups. The peak at 3025.67 cm^{-1} assigned the C-H stretching vibration of sp^2 hydrocarbon. The peaks at 2928.54 $cm^{-1}$and 2849.06 cm-1 informed the asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbons. The peaks which appear at 1459.69 cm^{-1} and 1380.21 cm^{-1} are due to C-H bending vibration o methyl groups. The bands at 1168.27 cm^{-1} and 1503.48 cm^{-1} show the C-C-O stretching vibration of alcohol group. The band which appears at 968.11 cm^{-1} should be C-H out of plane bending vibration of E- or trans- alkene group. Finally, the band at 794.44 cm^{-1} is due to the C-H out of plane bending vibration of Z- or cis- alkene group.The functional groups present in compound (4) are hydroxyl group, sp^2 hydrocarbons, sp^3 hydrocarbons, ether and Z- or cis- alkene groups from the FT- IR spectral data.

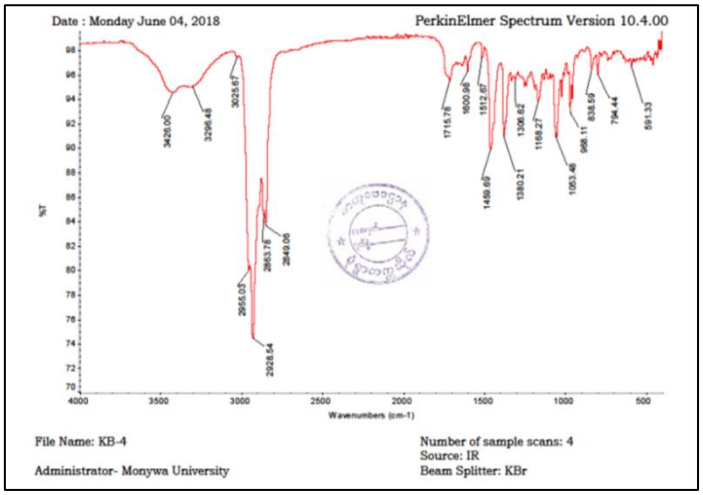

Fig. 5. FT-IR spectrum of isolated compound (4)

4 CONCLUSION

In this research, the whole plant of *Chromolaena odorata* L. was used to evaluate the phytochemical constituents and the mineral elements present in it. Phytochemical analysis of *Chromolaena odorata* L. showed the presence of alkaloids, phenolic compounds, glycosides, reducing sugar, tannins, saponins, polyphenols, steroids and flavonoids respectively. The antimicrobial activities of the whole plants of *Chromolaena odorata* L. were tested by Agar-well diffusion method. The ethanol extract showed medium activities on *Staphylococcus aureus* and *Candida albicans* and low activities on *Bacillus subtilis*, *Pseudomas aeruginosa*, *Bacillus pumilus* and *E.coli.* Ethyl acetate and n-hexane extracts show low activities on all selected organisms.

The antioxidant activity of ethanol extracts from the whole plant of *Chromolaena odorata* L. was determined in DPPH free radical scavenging assay. The IC_{50} value of the whole plant of *Chromolaena odorata* L. was found to be 381.003µg/ml and its activity is very low compared to that of standard ascorbic acid (IC_{50} 15.518µg/ml). The elemental compositions in the sample were determined using EDXRF spectrometer. According to EDXRF report, thirteen elements (Ca, Cl, K, Mg, Si, S, P, Fe, Mn, Zn, Ti, Cu, and Sr) were identified in the sample. Among them, calcium was found to be to the largest portion of the total elements and followed by chloride and potassium. This sample could be used as traditional medicine because of the lack of heavy toxic metal.

Furthermore, the pure compounds (1- 4) were isolated by using Thin Layer and Column Chromatographic separation methods. The yield percent of the isolated compounds were found to be 0.28% (11.2 mg), 0.4% (16.1 mg), 0.24% (9.5 mg) and 0.48% (19.5 mg) based on the ethyl acetate crude extract. The functional groups determinations of the compounds (1-4) were done by FT- IR spectral data. Compounds (1 and 2) contain alcohol and sp^3 hydrocarbons functional groups. Compounds (3 and 4) contain alcohol group, sp^3 hydrocarbons, sp^2 hydrocarbons and ether functional groups respectively.

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