A Potent Ingredient (*Plumbagorosea* Linn) of Myanmar Traditional Formulation Medicines

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Abstract

Plumbago rosea L. is an important medicinal plant, which grows in tropical regions of India. Samples were collected from traditional medicinal herbal shops in Chanayetharzan Township, Mandalay. After collection, the stem sample was washed, dried and ground. Then, the sample was extracted by methanol, ethanol and distilled water. The determination of phytochemical test, such as phenolic, glycoside, saponin, tannin, reducing sugar, terpene, flavonoid, steroid, lipophenol, polyphenol and alkaloid of the samples were studied by test tube method. Elemental constituents of the sample were determined by qualitative and quantitative analysis. The qualitative determination of the sample was done by energy dispersive X-ray fluorescence spectrophotometry. The quantitative determination of the sample was studied for toxicity by atomic absorption spectroscopy. Moreover, the antimicrobial activities of the sample were examined and the functional groups of isolated compounds were identified by FT-IR spectroscopy.

Keywords: antimicrobial activity, EDXRF, AAS, FTIR

Introduction

Plumbago rosea is an important medicinal plant, which grows in tropical regions of India (Komaraiah P. *et al*., 2004). Plumbaginaceae (*P.rosea*) have been reported by several researcher about the categorization of numerous compound namely plumbagic acid lactone cyanin and two aliphatics palmitic acid, myricyl palmitate, α- amyrin acetate, β-sitosterol, n-octacosanol, β-sitosterol, myricetin, roseanoic acid (Ragunathan M. *et al*., 2013). The stems of the plant are used in dyspepsia, chronic intermittent fever, ringworm, anemia, skin diseases, diarrhea, and abortifacient. The stem is extensively used in Siddha and Ayurveda medicine in India. The plant has been successfully applied to many medicinal and crop plants (Fulzele and Sative, 2003).

Materials and Methods

The plant of *Plumbago rosea* L. was collected from traditional medicinal herbal shop in Chanayetharzan Township, Mandalay. The sample was washed, dried and ground. The phytochemical screening of the sample was done by test tube method.

Determination of Elemental Content

Qualitative determination for elemental contents of the sample was studied at Monywa University by applying Energy Dispersive X-ray Fluorescence (SHIMAVZU-Model-EDX-7000) method. Quantitative determination for elemental contents of the plant was measured at Universities of Research Center, University of Yangon by applying Atomic Absorption Spectroscopy method.

Determination of Antimicrobial Activities

The antimicrobial activities of various extracts of sample were done by Agar well diffusion method on six selected organisms in Central Research and Development Centre (CRDC) Insein, Yangon. The selected organisms are *Bacillus*

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subtilis, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican*, and *E-coli.*

Separation of the Compounds by Column Chromatography

The sample (50 g) was percolated with Pet-ether (400 mL) for 8 hours soxhlet extract. The crude extract obtained from the stem of sample (1 g) was separated by column chromatography. The column was filled with gel slurry that was mixture of (20 g) silica gel and (120 mL) n-hexane. Then, the concentrated crude extract was dissolved in a minimum amount of EtOAc and introduced into the column along the wall by the use of a micro pipette. When the sample was reached the adsorbent, pure sand was added on the solute to obtain a sand layer of 1cm in thickness. The eluting solvent (n-hexane) was poured into the column. As the layer began to separate and move down the column, a small dry bottle was placed under the tap of the column to collect the eluent. About (2 mL) of each fraction was collected in small bottles by eluting solvent successively in the order as mentioned above 112 fractions were collected.

Separation of the Crude Sample by TLC

Each fraction was checked by TLC using various solvent system of n-hexane and EtOAc (9:1, 4:1, 7:3, 3:2, 1:1). Three isolated compounds were obtained. Each isolated compound solution was marked on the base line of TLC plate by using a fine capillary tube. The plate was then placed in the various solvent systems that was filled in TLC tank and covered with glass plate. The solvent was allowed to ascend and after the desired time of development, the plate was removed from the tank, and dried at room temperature. Then this plate was located with iodine vapor and more than one spot was observed in a yellowish background.

Identification of the Combined Fraction by TLC

From the TLC chromatograms, only one spot was found at fractions (19 to 26), (41 to 45) and fractions (66 to 67). Fractions with the same R_f values were combined and name as compound I, II, and III, and then checked by TLC plate with n-hexane: EtOAc.

Results and Discussion

From the study of the phytochemical test, it was found that, the samples contained of phenolic, glycoside, saponin, tannin, reducing sugar, lipophenol, terpene, flavonoid, steroid, polyphenol and alkaloid.

Elemental Contents of *Plumbago rosea* Linn

Elemental constituent of the samples was determined by qualitative and quantitative analysis. For the qualitative determination, the samples were measured by EDXRF (SHIMAVZU-Model-EDX-7000). The quantitative of the samples were examined by AAS method. From the qualitative determination of elemental contents of *Plumbago rosea* L., it was known that 0.892 % of potassium, 0.577 % of chlorine, 0.222 % of calcium, 0.220 % of sulfur, 0.186 % of silicon, 0.139 % of phosphorus, 0.020 % of iron, 0.002 % of manganese, 0.002 % of copper,0.002 % of titanium, 0.001 % of zinc and 0.001 % of bromine include in the sample. Among these elements, the amount of potassium was the highest percent in these samples. For quantitative determination, three toxic elements such as cadmium, lead and arsenic were studied by AAS method. The results were 0.018 mg of cadmium and 0.008 mg of arsenic in the sample. However, the content of lead was beyond the detection limit. According to FAO and WHO standard, the content of maximum permissible limit for person a day was found 0.01-0.45 mg/kg of cadmium, 0.01-0.43 mg/kg of lead and 0.02-1.20 mg/kg of arsenic. Therefore, the amounts of toxic

elements in the sample were lower than the FAO and WHO standard. (FAO/WHO, 2006)

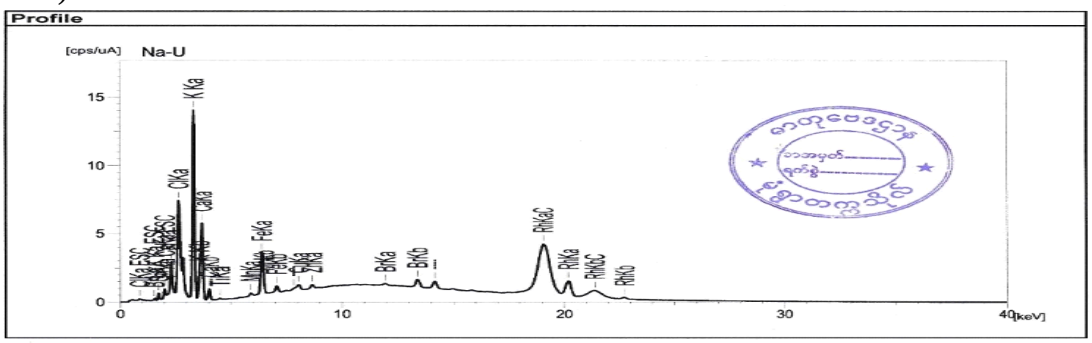

Figure (1) EDXRF spectrum of *Plumbago rosea* Linn

Determination of Antimicrobial Activity

The antimicrobial activities of the extracted sample were tested by applying Agar-well diffusion method on six selected organisms such *as Bacillus sublilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilius*, *candida albican* and *E-coli* species. The inhibition zones of various extracts are shown in Table 1. It was clearly noted that methanol, ethanol and ethyl acetate extract of sample possessed antimicrobial activity against all strains tested. Especially, ethanol extract was more active against *Staphylococcus aureus* and *Bacillus pumilus* species then others. Therefore, ethanol extract possessed antimicrobial activity.

Table (1) Antimicrobial Activities of *Plumbago rosea* Linn.

Sample	Solvents	Organisms					
		B-sub	S-aurens	Pseido-monas	B-pumalis	Candida	E-coil
Plumbago rosea Linn	MeOH	13 mm (+)	12 mm (+)	12 mm (+)	11 mm (+)	11 mm (+)	11 mm (+)
	EtOAc	11 mm (+)	12 mm (+)	12 mm (+)	13 mm (+)	14 mm (+)	13 mm (+)
	EtOH	14 mm (+)	17 mm (++)	14 mm (+)	18 mm (++)	14 mm (+)	14 mm (+)

Agar well – 10 mm 1.*Bacillus subtilis*(N.C.T.C.8236)

2. *Staphylococcus aureus* (N.C.P.C.-6371)3. *Pseudomona aeruginosa*(6749)

4. *Bacillus pumilus*(N.C.I.B-8982)5. *Candida albican*

6. *E-coil* (N.C.I.B-8134)

Identification of the Compounds

The three isolated compounds of Pet-ether extract were measured by FT-IR spectrophotometry method at the Department of Chemistry, Monywa University. The R_f value of compounds I, II and III were 0.560, 0.414 and 0.536 respectively.

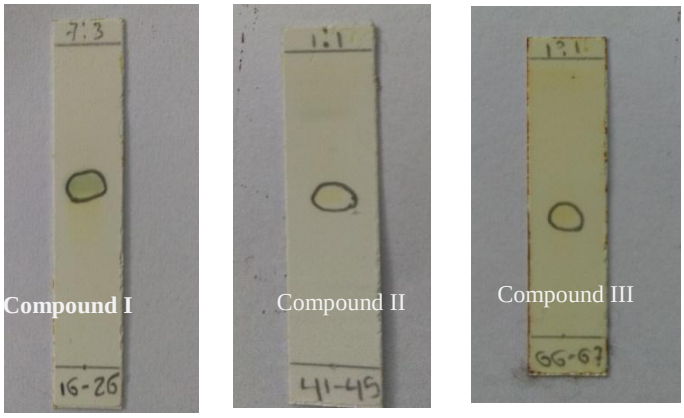

Figure (2) TLC of isolated compound I, II and III

FT IR Assignment of Isolated Compounds

In the spectrum of the compound I, the band with appears at 3393.75 cm^{-1} represented the O-H stretching vibration of alcohol group. The sharp peaks at 2924.31 cm^{-1} and 2853.88 cm^{-1} indicated the asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbon. The existence of C=O stretching vibration of carbonyl group could be observed at 1710.87 cm^{-1}. The peak at 1640.41 cm^{-1} implied the C=C stretching vibration of aromatic benzene ring. The peak at 1455.39 cm^{-1} and 1376.07 cm^{-1} represented the C-H in plane bending vibration of an allylic hydrocarbon. The peak at 1241.51 cm^{-1} and 1215.61 cm^{-1} showed the C-C-O stretching vibration of alcohol group. The ether functional group could be observed at 1163.64 cm^{-1}, 1089.79 cm^{-1} and 1045.19 cm^{-1}. The peaks at 797.13 cm^{-1} and 904.24 cm^{-1} showed C-H out of plane bending vibration of trans or E and the peaks at 785.85 cm^{-1} and 744.88 cm^{-1} indicated C-H out of plane bending vibration of cis or Z alkenic groups. According to this spectral data, compound I contained alcohol group, sp^3 hydrocarbon, carbonyl group, aromatic benzene ring, allylic hydrocarbon, ether group and trans or E and cis or z alkenic groups respectively.

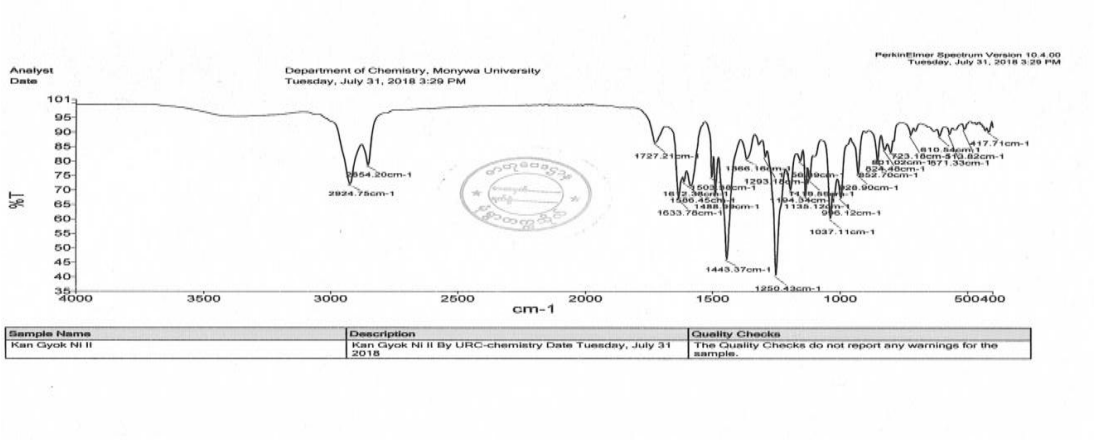

Figure (3) FT IR spectrum of compound I

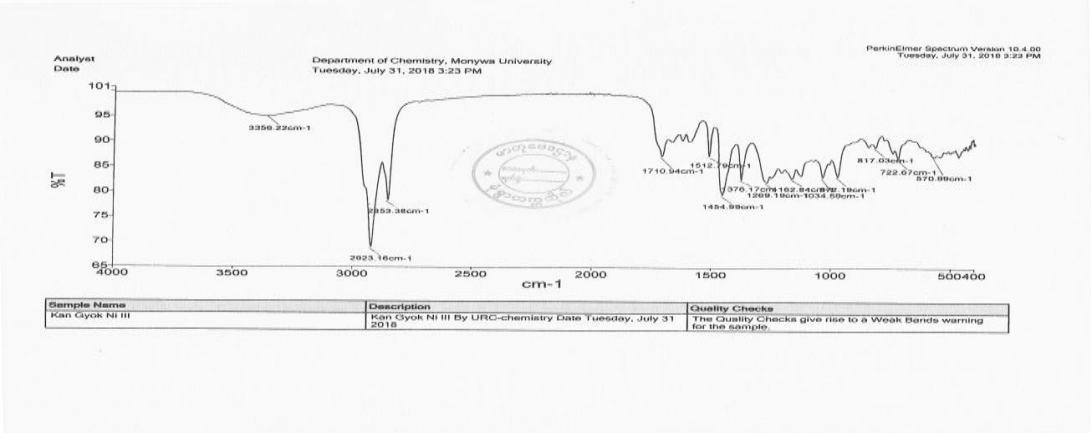

Figure (3) FT IR spectrum of compound II

Similarly, in the FT-IR spectrum of compound II, isolated compound-II contained sp^3 hydrocarbon, carbonyl group, aromatic benzene ring, allylic hydrocarbon, ether group and

trans or E and cis or Z alkenic group and the compound-III spectrum represented the O-H stretching vibration of alcohol group, the asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbon, C=O stretching vibration of carbonyl group, C=C stretching vibration of alkanic group, the C-H in plane bending vibration of allylic hydrocarbon, the C-C-O stretching vibration of alcohol group, ether functional group, C-H out of plane bending vibration of trans or E and cis or Z alkanic groups respectively. According to literature, it was recognized that plumbagin contained in the *Plumbago rosea* Linn. The functional groups of plumbagin were contained alcohol group, sp^3 hydrocarbon, carbonyl group, aromatic benzene ring and ether group respectively. It suggested that compound I, II and III might be present derivative compounds of plumbagin.

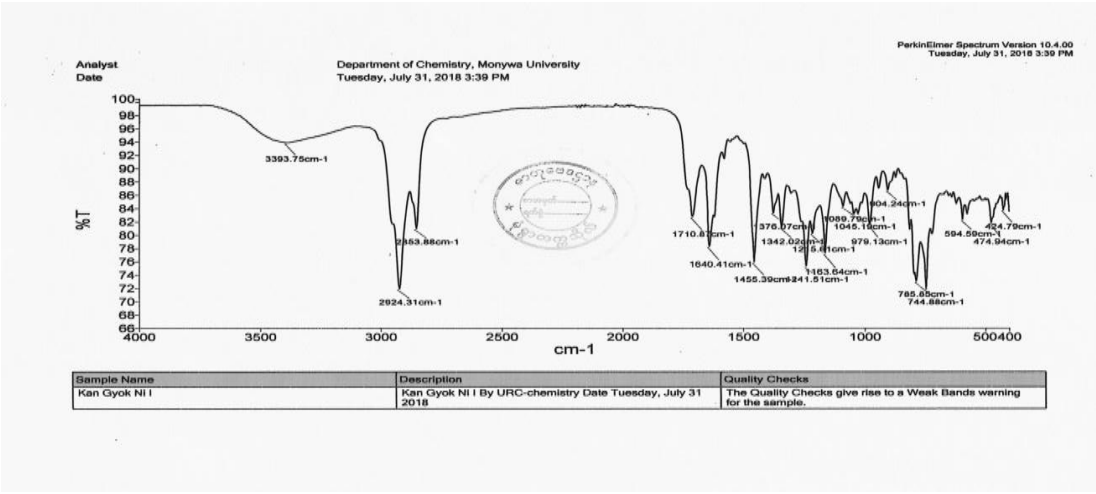

Figure (4) FT IR spectrum of compound III

Conclusion

The samples were collected from traditional herbal shop, Chanayetharzan Township, Mandalay. It was found that, the sample consists of phenolic, glycoside, saponin, tannin, reducing sugar, lipophenol, flavonoid, polyphenol compounds and steroid.

From the qualitative determination of elemental contents of *Plumbago rosea* L., it was known that the sample includes 0.892 % of potassium, 0.577 % of

chlorine, 0.222 % of calcium, 0.220 % of sulfur, 0.186 % of silicon, 0.139 % of phosphorus, 0.020 % of iron, 0.002 % of manganese, 0.002 % of copper, 0.002 % of titanium, 0.001 % of zinc and 0.001 % of bromine . Among these elements, the amount of potassium was the highest percent in these samples.

For quantitative determination, three toxic elements such as cadmium, lead and arsenic were studied by AAS method. The results were 0.017 mg, 0.018 mg of cadmium and 0.019 mg, 0.008 mg of arsenic in the samples. However, the content of lead was beyond the detection limit. According to FAO and WHO standard, the content of maximum permissible limit for person a day was found 0.01- 0.45 mg/kg of cadmium, 0.01-0.43 mg/kg of lead and 0.02-1.20 mg/kg of arsenic. Therefore, the amount of toxic elements in the sample was lower than the FAO and WHO permissible limit of heavy elements.

The antimicrobial activities of the stem of the sample were tested by Agar-well diffusion method on six tested organisms such as *Bacillus subtilis*, *Staphylococcus aureas*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *E.coil* respectively. It is clearly noted that methanol, ethanol and ethyl acetate extract of sample possessed antimicrobial activity against all strains tested. Especially, ethanol extract was more active against *Staphylococcus aureus* and *Bacillus pumilus* species then others. Therefore, ethanol extract possessed antimicrobial activity.

According to FT-IR spectral data, compound I, II and III was contained – OH group, sp^3 hydrocarbon, carbonyl group, aromatic benzene rings, allylic hydrocarbon, alcohol group, ether groups, alkene groups respectively.

To conclude, the *Plumbago rosea* L. is composed in the formulation of Myanmar Traditional Medicines; TMF- 12, TMF-23, TMF-24, TMF-27, TMF-31, TMF-34, TMF-35 (a), TMF-35 (b), TMF-37, TMF-38 and TMF-40. Moreover, it possessed twelve elements and the three toxic elements that were under the maximum permissible level, antioxidant activity and antimicrobial activity. Therefore, the stem of *Plumbago rosea* L. is a safe and harmless ingredient for medication.

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