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Isolation and Characterization of Flavonoids from Chloroxylon Swietenia

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ABSTRACT

The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline some where in between natural products (organic chemistry) and plant biochemistry and is related to both. The Flavonoids are polyphenolic compounds possessing 15 carbon atoms and two benzene rings joined by linear three carbon chain. This occur in most plant species and accounts for significant percentage of chemical constituents. Flavonoid compounds are isolated from the flowers of local medicinal plant like Chloroxylon Swietenia flowers. On the basis of chemical and spectral analyses their structures are elucidated as Gossypetin -8-O-β-D glucopyronoside 3- Sulphate. It is known that the compound was isolated from this plant for the first time. Identification of unknown compound was established through comparison of spectral data (UV, IR, and NMR) with literature values.

Key words: Porasu or Mammarai (Tamil) flower, Benzene, Ethanol, Isolation, flavonoids, Ethyl acetate fraction, H^1 Spectrum and C^{13} Spectrum.

INTRODUCTION

Nature produces a large number of chemical compounds whose structures and properties have fascinated organic chemists who have evolved a language of their own to describe the chemistry of these compounds[1].It is concerned with enormous variety or organic substances namely alkaloids, caratenoids, steroids, flavonoids, terphenoids, etc. are elaborated and accumulated by plants and deals with the chemical structures of these substances the biosynthesis turn and metabolism natural distribution and their biological function[2].Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognized

as flower figments in most angiosperm families (flowering pigments). The Rutaceae accounting for about 100 genera and 800 species[7] is a family which contains a variety of unusual and highly substituted flavonoids. The citrus is the most widely studied, genus of this family. The Flavonoids are isolated from the flowers of local plant like Chloroxylon Swietenia fig (1). Which is belongs to Rutaceae family. Chloroxylon Swietenia is popularly known as Porasu or Mammarai in Tamil. The park of the tree is used as an astringent, its leaves are applied to wounds and it is also prescribed in rheumatism. It is distributed in the Indian peninsula, extending in the north to the Satpuras and Chotanagpur.

EXPERIMENTAL SECTION

Extraction and Fractionation

Fresh flowers of Chloroxylon Swietenia collected from Komapuram, Gandarvakkottai (T.K) Pudukkotai (DT) Tamil Nadu, India, during March. It is extracted with 90% ethanol (4×500 ml) under reflux. The alcoholic extract is concentrated invacuo and the aqueous concentrate successively fractionated with benzene (3×250 ml) peroxide free Et_2O (3×250ml) and EtOAC (4×250 ml). The benzene fraction does not yield any isolable material.

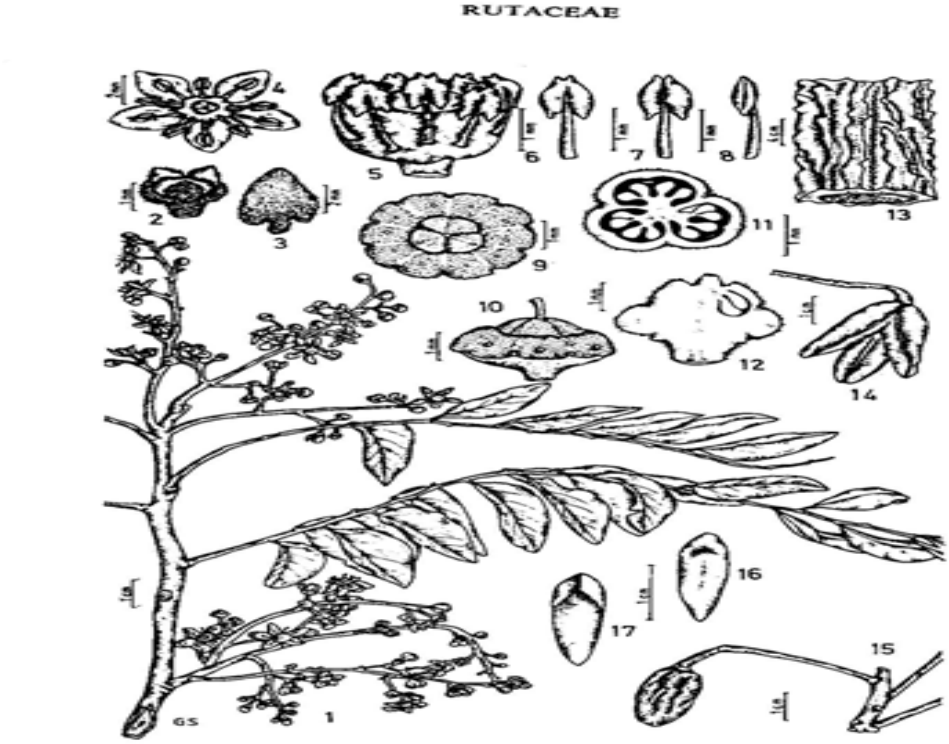

PLATE 103 **Chloroxylon swietenia** DC. **1** twig ; **2** calyx ; **3** petal ; **4** flower ; **5** staminal cluster ; **6-8** stamens ; **9** disc ; **10** pistil with disc ; **11 & 12** pistil, t.s. & l.s. ; **13** bark ; **14 & 15** capsule, dehisced & entire ; **16 & 17** seeds. (**1** from **RHT** 27116 ; **13** from **RHT** 16849 ; **15** from **RHT** 7745 ; **14, 16 & 17** from **RHT** 2693 ; others from **RHT** 1060)

Fig (1) Chloroxylon Swietenia flowers and its parts

Diethyl ether (Et_2O) fraction (Flavone, Gossypetin)

The yellow solid isolated from Et_2O fraction yielded, yellow crystals M.P.313-315^oC on recrystallisation with MeOH. It is soluble in organic solvents and sparingly in hot water. It will give a red colour with Mg/HCl, green colour with Alc.F^{3+} yellow colour with NH_3 and NaOH and yellow solution with conc. H_2SO_4. It appeared yellow under UV with or without NH_3. It responded to Horhammer Hansal[21] and Wilson boric acid tests[21]. It never answers responds to the Gibb's31 and molisch test. It had λ MeOH nm 261,276,309,339,385; +NaOMe-251,287,366(dec); + $AlCl_3$290, 327, 401,472 + AlCl3/HCl -274, 292 $_{sh}$, // 313,373,372,447; + NaOAc-281,366(dec); +NaOAc /H_3BO_3 -273, 282, $_{sh}$, 314 $_{sh}$ 358,396. It had RF values as depicted in table1.Rf(X100) values of the constituents of the flowers of Chloroxylon Swietenia.

Table 1.0 Rf(X100) values of the constituents of the flowers of Chloroxylon Swietenia

Compound	Developing solvents								
	a	b	c	d	e	f	g	h	i
Aglycone from Et_2O fraction	-	02	04	10	20	19	17	28	48
Gossypetin	-	02	04	10	20	19	17	28	48
Gossypetin (authentic)	-	20	05	9	20	45	65	75	25 60
glycoside from EtOAC fraction									

Ethyl Acetate (EtOAC) Fraction :(Gossypetin -8-O-β-D glucopyronoside 3- Sulphate)

EtOAC fraction was concentrated invacuo and left in an ice chest for a day when a yellow solid separated which was filtered and studied when crystallized from MeOH in came out as yellow long needles. It was freely soluble in aq NaOH hot water, EtOH and EtOAC but insoluble in $Et_2O$and $CHCl_3$it appeared yellow under UV. Which terms deep yellow and fuming ammonia it respond to answered Wilson's boric acid. Gibbs and malisch test did not respond to Horhammer Hansel test. It had λ_{Max} MeOH nm 258,270 sh, 365,sh, 379+NaOMe 245sh, 295sh,331,428sh (dec);+ $AlCl_3$260sh,275,309sh,364sh,452; +$AlCl_3$+HCl 269, 307 Sh,367,441; + NaOAc 278, 328, 400(dec); + NaOAc-H_3BO_3 -267, 277, sh, 325 sh, 400. It had RF values as depicte in table1.

Hydrolysis of the Glycoside

The glycoside was dissolved in hot aq MeOH (2ml 5%) and an equal volume of H_2SO_4 7% was added to it the reaction mixture was refluxed under 100^o C for 2 hours. The excess of alcohol was distilled off from the hydrolysate and the resulting aq. Solution was diluted with more water and left and chilled conditions for 2 hours the extract was fractioned with Et_2O.

Identification of the aglycone

The residue from ether fraction of the hydrolysate was taken up in AC_2O and left and child conditions for few days, yellow colour solid was obtained and its colour reactions chromatographic behavior and UV spectral data ascertained, compound has been ascertained as Gossypetin 3- Sulphate. The presence of sulphur has been found by heating the solution of the compound obtained from sodium fusion test purple colour with freshly prepared sodium nitroprusside.

Identification of sugar (Glucose)

The aqueous hydrolysate after the removal of the aglycone was cautiously neutralized with barium carbonate and filtered. The concentrated filtrate on PC gave RF values corresponding to these of glucose. The running properties of the glycoside were infavour of a monoside. The identified of the sugar was also confirmed with an authentic sample of glucose.

Anti- microbial activity of flavonoids

A number of flavonoids too have been shown to have antimicrobial activity. Several viruses [29] and bacteria are sensitive to quercetin. Coumarins have been reported to possess activity against many bacteria and fungi[30]**.** Leaves of Didymocarpus spp. have been reported to possess antimicrobial activity and those contain many flavonoids.[31]**.** Dyhydrochalcones and flavones obtained from Uvaria angolensis have been to contain antibacterial activity[32]**.** The antimicrobial activity of many flavonoids has been recorded [33]**.** The popular methods of testing antimicrobial activity of a compound are (i) disc diffusion technique[34], (ii) serial dilution technique[35] and ditch plate technique[36]**.** When a bacterial organism is allowed to grow in a broth culture medium, becomes turbid due to multiplication of bacterial cells and formation of bacterial colonies. The turbidity is a measure of bacterial growth. A potent drug should inhibit the bacterial growth and hence the turbidity formation. This forms the basis for testing the antimicrobial activity of a drug by the serial dilution technique. Enterobactor sp. of the gram negative group and Staphylococcus sp. of the gram positive group were chosen as test organism fig (1) and fig (1a). The MH agar plate was divided into four sections and smeared each with pure flavones solution of dilution 1/2, 1/4 1/8 on an area equivalent to 400 mm^2. It was incubated for 18 hours. The two controls Enterobactor sp and Staphylococcus sp were smeared to 4 different areas equivalent to 400 mm^2

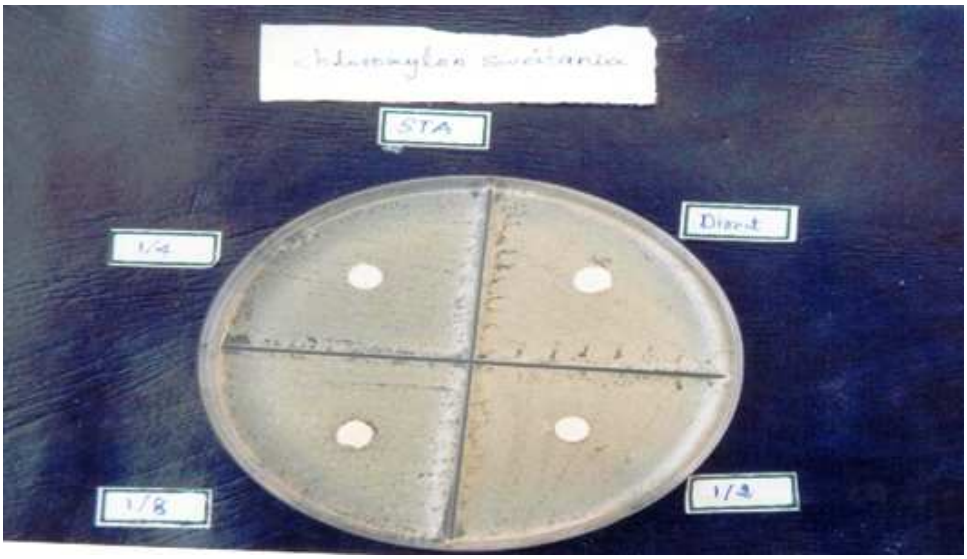

Fig (1) Anti Bacterial Activity of Chloroxylon Swietenia

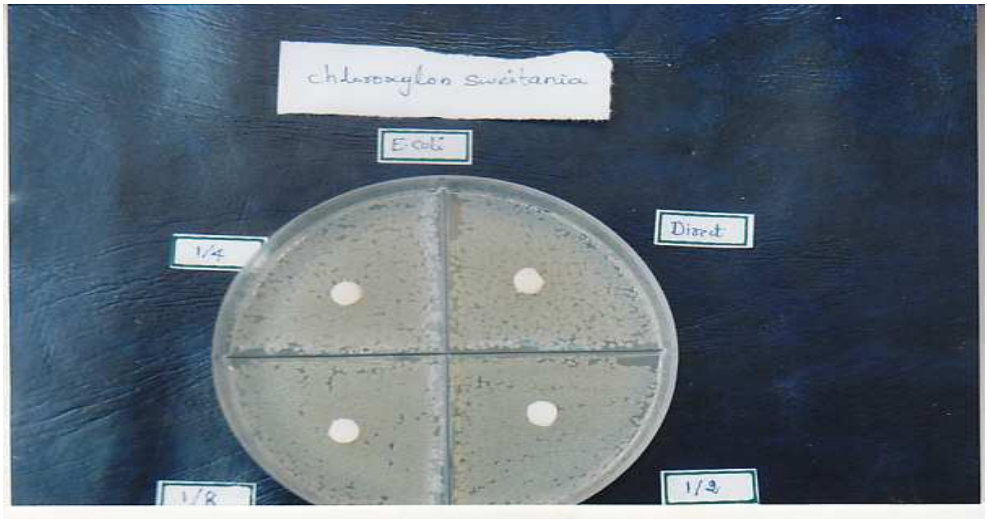

Fig (1a) Anti Bacterial Activity of Chloroxylon Swietenia

RESULTS AND DISCUSSION

The flowers of C. Sweitenia have been found to contain Gossypetin and its glycoside Gossypetin-8-O-β-D glucopyronoside 3- Sulphate, fig(3) the UV spectra of flavonol aglycone obtained from the Et_2O fraction exhibited two major peaks at 385 nm (band-I) and two 261 nm (band-II) which showed a flavonol skeleton .The spectrum get degenerated on the addition of NaOMe which indicate the presence of a 3', 4' dihydroxy grouping a bathochromic shift of 62 nm on the addition of $AlCl_3$ –HCl showed the presence of a free 5OH in the A ring. The presence of a free OH at C7 was ascertained by a shift of +20 nm (band II) on the addition of NaOAc. The AlCl3 spectrum showed the bathochromic shift of +25 nm over and above that of AlCl3 –HCl spectrum ascertained the presence of the catechol type of O- dihydroxy grouping in B as well as A- rings. The boric acid spectrum also proved it. The structure of the aglycone has been established as Gossypetin by means of chemical reactions, RF values and hydrolytic studies. The glycoside from EtOAC fraction had max at 379 nm (band I) and 258 nm(band II)suggesting a flavonol skeleton it would be hydrolyzed 7% H_2SO_4 100^o C 2h.) to Gossypetin- 3- sulphate and glucose the presence of free OH at C_5 in the glycoside on the aglycone in evident from it responds to Wilson boric acid test, the same observation also stems from the fact that a bathochromic shift of 62 nm could be observed in the glycoside in the $AlCl_3$-HCl spectra. A bathochromic shift of 49 nm in the glycoside noticed on the addition of NaOMe cause a bathochromic shift of 20nm (band II) ascertained a free OH at C_7.

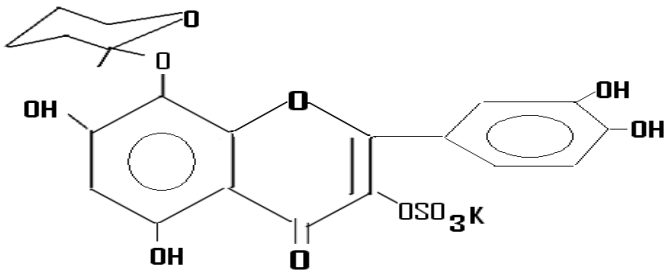

Fig (3) Structure of Gossypetin-8-O-β-D glucopyronoside 3- Sulphate

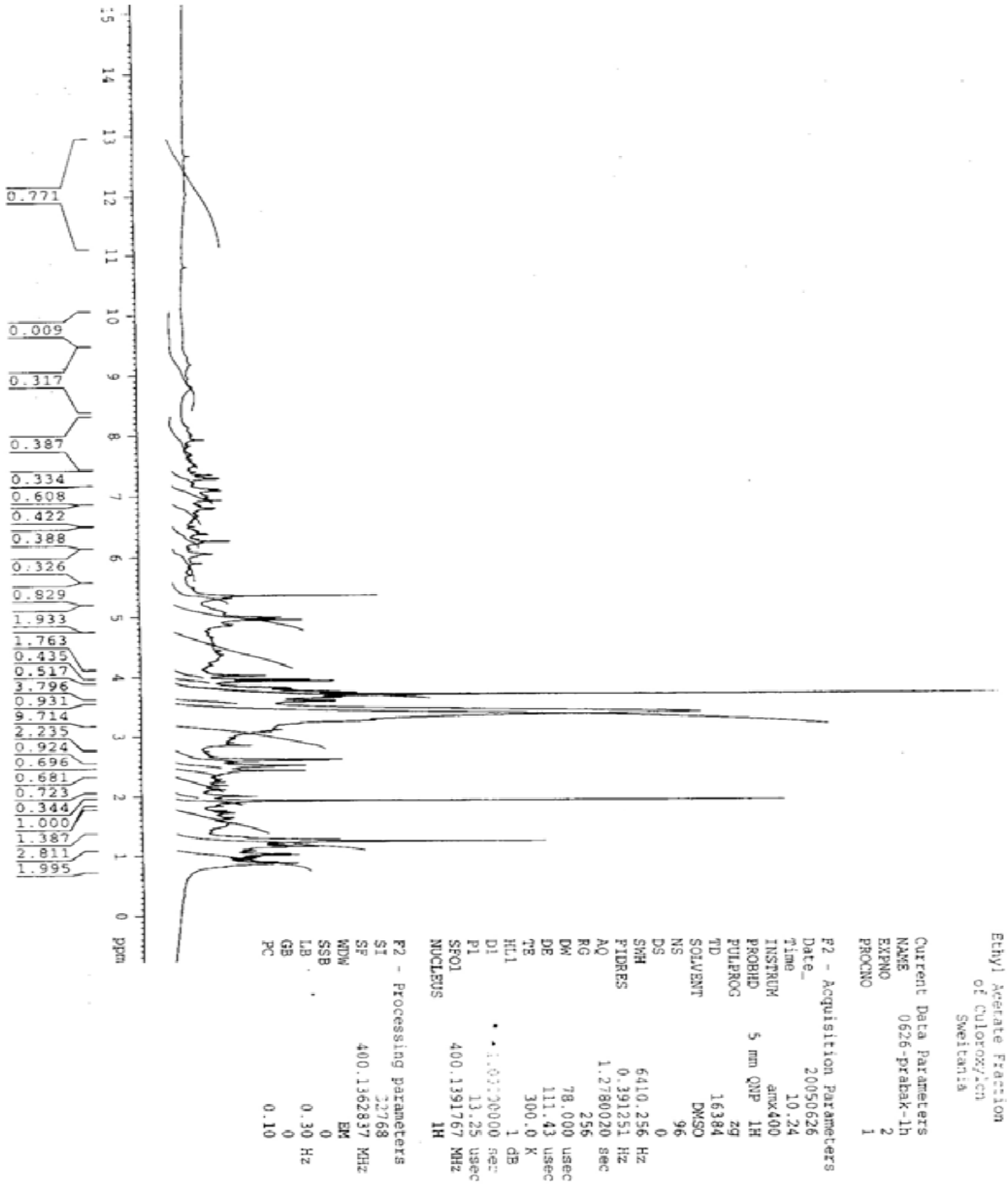

Fig (4) ^{1}H-NMR Spectrum EtOAC Fraction of Chlroxylon Sweitenia

In the ^{1}H- NMR Spectrum 400 MHz DMSO – d6, TMS) fig (4) of the flavonol glycoside the C_5 proton is strongly deshielded by 4 keto group and appears at δ12.7 ppm. The free 7 OH group has been evidence from a resonating peak at δ10.8 ppm the proton at C_6 so up at δ6.92 ppm. The $C_5{}^{1}$ appears as a doublet at δ7.34 ppm and 7.91 ppm – respectively. the C1 proton of the terminal sugar (H-1'') being relatively removed from the influence of the flavonoid nucleus, resonates at

δ4.98 ppm the rest of the sugar protons appear at 3.38 ppm supporting evidence for the structure of the glycoside was provided by the analysis of ^{13} C NMR (100 MHz DMSO – d6, TMS) fig (5) data and complete assignment is given (table II- 1).

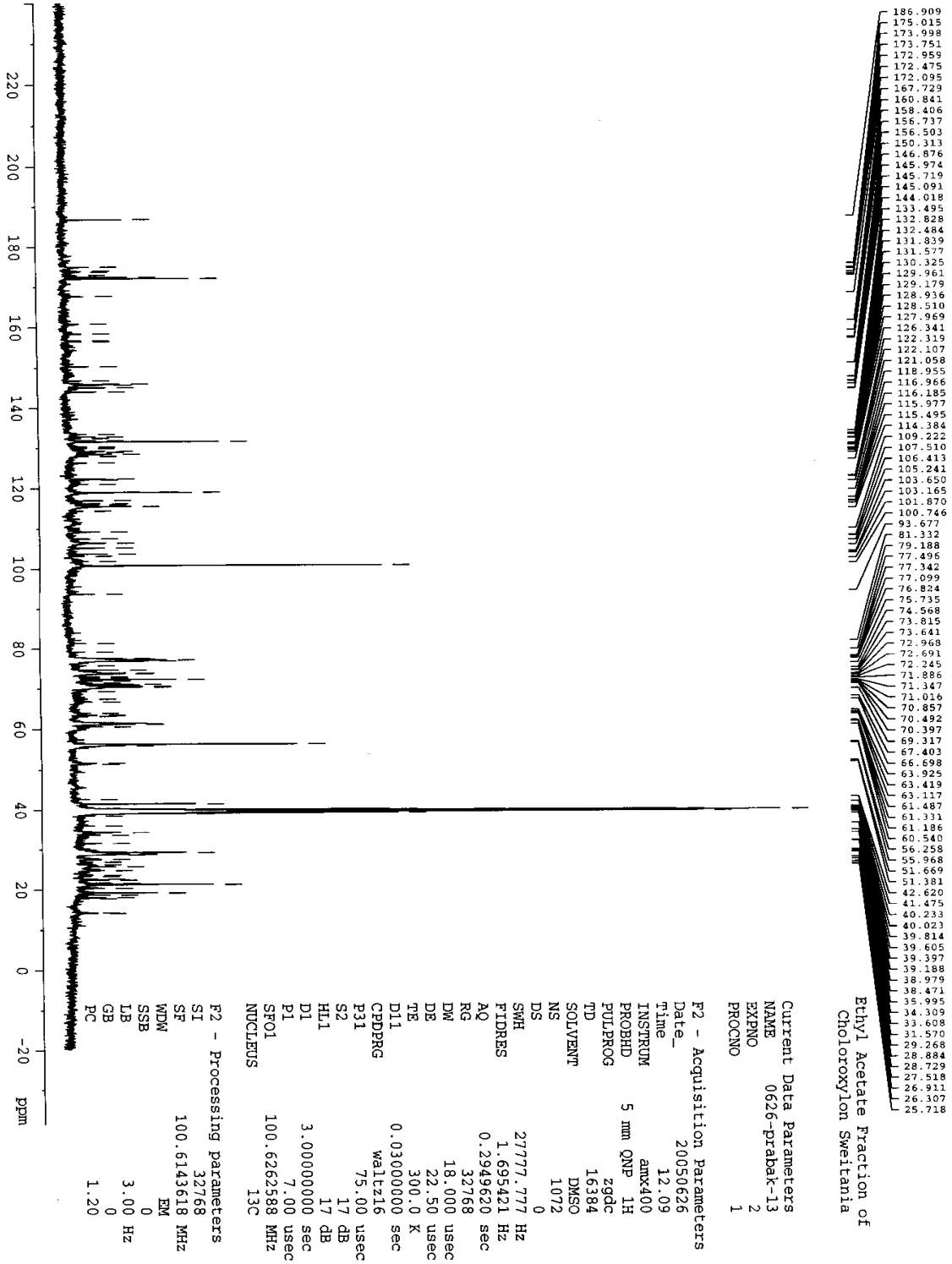

Fig (5) ^{13}C-NMR spectrum EtOAC Fraction of Chlroxylon Sweitenia

Based on this the glycoside has been characterized as Gossypetin -8-O-β-D- Glucopyronoside-3-Sulphate.Antibacterial property of the flavones used to have normally either bacteriostatic or bacteriocidal in nature. To our dismay that the glycoside isolated from C.swietenia where unable to on the chosen, gram positive organism, staphylococcus aureus and the gram negative organism, E. coli. Even at the highest dosage of 1000µg. the yellow pigments namely Gossypetin -8-O-β- D glucopyronoside 3- sulphate from the plant is inactive.

CONCLUSION

It deals with the studies on the isolation and characterization of flavonoids from the flower of Chloroxylon Swietenia respectively using UV, NMR and chromatographic techniques, Chloroxylon Swietenia have been found to contain Gossypetin -8-O-β-D- glucopyronoside-3-sulphate respectively. These results suggest the compounds have excellent scope for further development as antimicrobial activity.

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