

Investigation of the Medicinal Potentials of *Syzygium jambos* (L.) Extract and Characterization of the Isolated Compounds

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Abstract: *Syzygium jambos* (L.) Alstone belonging to the Myrtaceae family have been investigated for isolation of their secondary metabolites and evaluation of their biological activities. A total of three compounds have been isolated. The isolated compounds were identified by extensive analysis of their high resolution 1H-NMR spectroscopic data. The dried stem barks of *Syzygium jambos* was extracted with methanol. The concentrated methanolic extract was then partitioned with vacuum liquid chromatography. The first investigation was conducted with the VLC fraction-6 afforded one pentacyclic triterpenes (SJ-61), VLC fraction-9 afforded a sitosterol (SJ-92) and VLC fraction-16 afforded a triterpene 3-nor-2,3-fridelan derivative (SJ-184). In the brine shrimp lethality bioassay, The LC₅₀ values were found to be 6.97, 18.07, 64.943, 247.596 and 12.75µg/ml for crude methanolic extract, carbon tetrachloride, chloroform and *n*-hexane soluble fractions of methanolic extract of *Syzygium jambos* respectively representing methanolic extract fraction as highly cytotoxic.

Keywords: Medicinal Plant, Extraction, Isolation, Characterization, Cytotoxicity, Biological Activity

1. Introduction

In the plant kingdom there is a remedy for every disease^[1]. Two hundred and fifty years ago, there were few or no synthetic medicines. The plants were the main source of drugs for the world's population. Today, 75% of the world's population, the poor 3/4ths, still relies on those plants and other tools of traditional medicine^[2]. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being^[3].

The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000^[4], with a lower level at 215,000^[5] and an upper level as high as 500,000^[6,7]. Of these, only about 6% have been screened for biologic activity, and a reported 15% have been evaluated phytochemically^[8]. Chemical diversity of secondary plant metabolites that results from plant evolution may be equal or superior to that found in synthetic combinatorial chemical libraries. Success in natural products research is conditioned by a careful plant selection, based on various criteria such as chemotaxonomic data, ethnomedical information, field

observations or even random collection. One main strategy in the isolation of new leads consists of the so-called bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive compounds^[9].

The work described in this dissertation is an attempt to isolate and characterize the chemical constituents of medicinal plants, viz., *Syzygium jambos* (Fam. Myrtaceae) and to evaluate the possible cytotoxic profiles of the crude extracts

2. Materials and Methods

Collection and preparation of the plant (*Syzygium jambos*) material: Plant sample of *Syzygium jambos* were collected from Gazipur, Bangladesh and was identified by an expert taxonomist of Botany Department, University of Dhaka (plant reference no. is DUSH7179). The stem barks were separated, cut into small pieces, sun dried for several days followed by oven dried for 24 hours at 40° C to facilitate grinding and finally crushed into course powder.

Extraction and fractionation: About 200gram of powdered stem barks in methanol for two weeks with occasional shaking. The mixture was filtered, concentrated at 39°C to have a concentrated crude extract. 5gram of the extract was subjected to solvent-solvent partition for investigation of the biological activities and the remaining amount was used for chemical investigation^[10,11]. The concentrated methanol extract (5 g) was fractionated by modified Kupchan method^[12] and the resultant partitionates i.e., petroleum ether, carbon tetrachloride, chloroform and aqueous soluble materials were used for different biological screenings.

2.1. Chemical Investigation of the Purified Crude Extract of *S. jambos*

The methanol extract was subjected to extensive chromatographic separation. The isolated pure compounds were then characterized by extensive spectroscopic studies like proton NMR techniques, using CDCl₃ as solvent.

2.2. Brine Shrimp Lethality Assay of the Purified Crude Extract of *S. jambos*

This technique^[13] was applied for the determination of general toxic property of the plant extractives indicating bioactive materials using *Artemia salina* in a 1-day in vivo assay. Vincristine sulfate was used as positive control. Brine shrimp lethality bioassay^[14] is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin and is considered a useful tool for preliminary assessment of toxicity.

Brine shrimp toxicity is closely correlated with 9KB (human nasopharyngeal carcinoma) cytotoxicity (p=0.036 and kappa = 0.56). ED₅₀ values for cytotoxicities are generally about one-tenth the LC₅₀ values found in the brine shrimp test. Thus, it is possible to detect and then monitor the fractionation of cytotoxic, as well as 3PS (P388) (in vivo

murine leukaemia) active extracts using the brine shrimp lethality bioassay^[11].

3. Results and Discussion of the Test Samples of *S. jambos*

Bioactive compounds are almost always toxic at higher dose. Thus, *in vivo* lethality in a simple zoological organism can be used as a convenient informant for screening and fractionation in the discovery of new bioactive natural products.

3.1. Chemical Investigation of the Purified Crude Extract of *S. Jambos*

One steroid and two triterpenes were yielded from the methanolic extracts of stem barks of *S. jambos*. Through repeated chromatography and purification, extensive spectroscopic technique (proton NMR techniques) was applied to determine the structure.

3.1.1. Characterization of SJ-61 as Friedelin

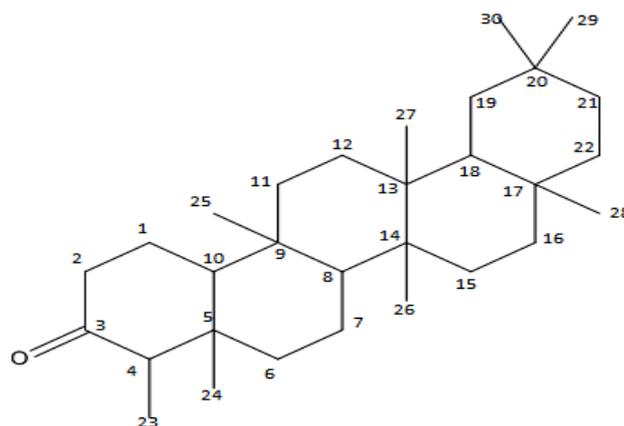


Figure 1. Compound SJ-61 (Friedelin)

Compound SJ-61 was obtained (Fig-1) from the VLC fraction 6 (15% ethyl acetate in hexane) of crude extract as white crystals. It was evident as a yellow spot on TLC (Silica gel PF₂₅₄) when the developed plate was sprayed with vanillin-sulfuric acid followed by heating at 110°C for 5-10 minutes. It was found to be soluble in petroleum ether, hexane, ethyl acetate and chloroform.

The ¹H NMR spectrum (500 MHz, CDCl₃) of SJ-61 (Figure-2 & table-1) revealed signal for two double doublet of proton intensity at δ 2.23 typical for H-2b and at δ 2.23 typical for H-1a (1H, dd J=13.0, 7.5 Hz). A one proton multiplet was found for H-4 of pentacyclic triterpenoid and one equatorial proton (H_{eq}-2) at δ 2.37. The spectrum displayed another multiplate at δ 1.96 for H-1a, originated from eight singlet signals in the regions 0.71 ppm, 0.86 ppm, 0.87 ppm, 0.94 ppm, 0.99 ppm, 1.00 ppm, 1.04 ppm and 1.17 ppm each integrating for three protons assignable to eight tertiary methyl groups at C-24, C-25, C-23, C-30, C-26, C-27, C-28 and C-29 respectively.

Table 1. Comparison between ^1H NMR spectral data of SJ-61 (500 MHz) and Friedelin in CDCl_3 (Dhaka Univ. J. Sci. 59(2): 179-180, 2011 (July)).

Protons	δ_{H} in ppm in CDCl_3	
	SJ-61	Friedelin
H-1a	1.96 (1H, m)	1.97 (1H, m)
H-2b	2.29 (1H, dd, 13.0, 7.5 Hz)	2.28 (1H dd 13.2, 7.2 Hz)
H-4	2.37 (1H, m)	2.40 (1H, m)
H ₃ -23	0.87 (3H, d, 7.5Hz)	0.89 (3H, d, J=7.2 Hz)
H ₃ -24	0.71 (3H, s)	0.74 (3H, s)
H ₃ -25	0.86 (3H, s)	0.88 (3H, s)
H ₃ -26	0.99 (3H, s)	1.01 (3H, s)
H ₃ -27	1.00 (3H, s)	1.02 (3H, s)
H ₃ -28	1.04 (3H, s)	1.06 (3H, s)
H ₃ -29	1.17 (3H, s)	1.19 (3H, s)

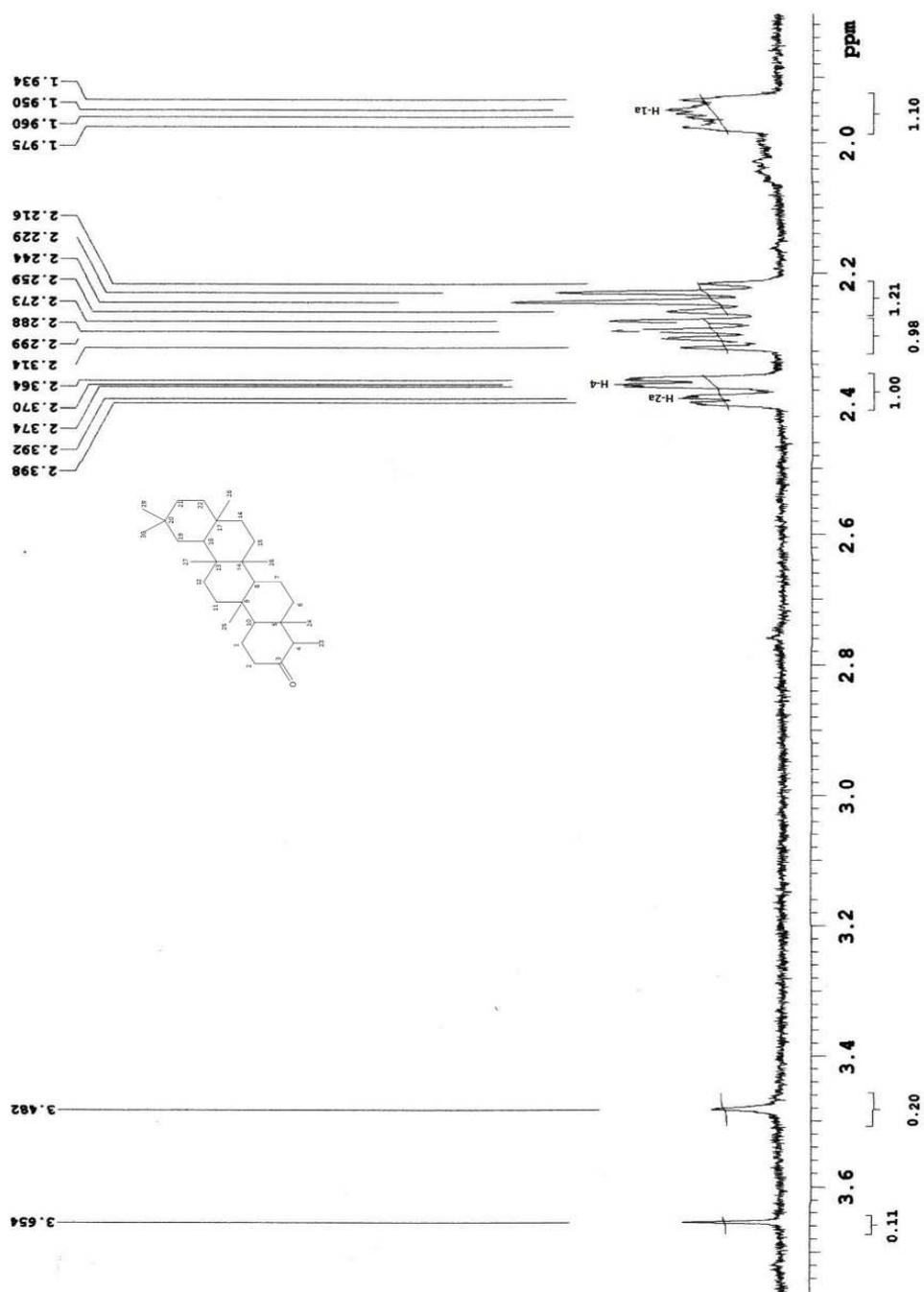
**Figure 2.** ^1H NMR of spectrum of SJ-61 in CDCl_3

Table 2. ¹H NMR spectral data of SJ-92 (500 MHz) and β-sitosterol (Dhaka Univ. J. Sci. 59(2): 179-180, 2011 (July) in CDCl₃.

Position	δ _H in ppm in CDCl ₃	
	SJ-92	β-sitosterol
H-3	5.34 (1H d, J = 5.2 Hz)	5.33 (1H d, J = 7.0 Hz)
H-6	3.52 (1H, m)	3.51 (1H, m)
H ₃ -18	0.67 (s)	0.67 (s)
H ₃ -19	1.00 (s)	1.00 (s)
H ₃ -21	0.91 (3H d, J = 6.4 Hz)	0.92 (3H d, J = 6.6 Hz)
H ₃ -26	0.84 (3H d, J = 7.2 Hz)	0.84 (3H d, J = 7.0 Hz)
H ₃ -27	0.82 (3H d, J = 7.2 Hz)	0.81 (3H d, J = 7.0 Hz)
H ₃ -29	0.85 (3H t, J = 6.0 Hz)	0.85 (3H, t, J = 7.0 Hz)

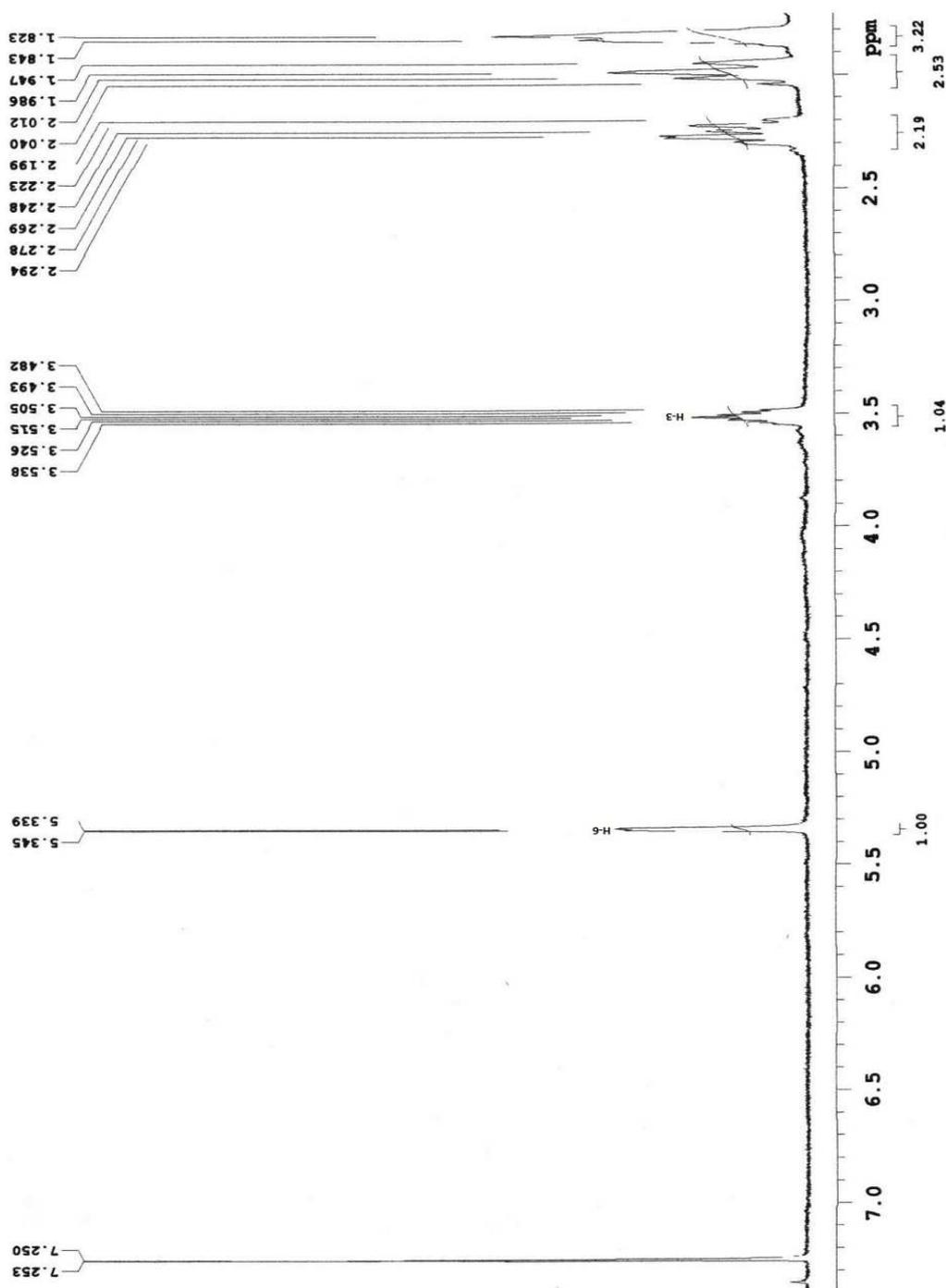


Figure 3. ¹H NMR spectrum of SJ-92 in CDCl₃

3.1.2. Characterization of SJ-92 as β -Sitosterol

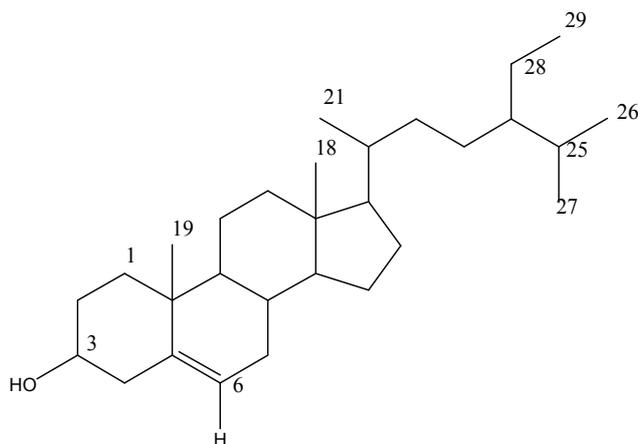


Figure 4. Compound SJ-92 (β -Sitosterol)

Compound SJ-92 was obtained as needle shaped crystals (Fig-4). It was evident as a purple spot on TLC (Silica gel PF₂₅₄) when the developed plate was sprayed with vanillin-sulfuric acid followed by heating at 110°C for 5-10 minutes. It was found to be soluble in petroleum ether, hexane, ethyl acetate and chloroform.

The ¹H NMR spectrum (500 MHz, CDCl₃) of SJ-92 (Figure-3 & Table-2) revealed the typical signal for the olefinic H-3 of the steroidal skeleton was evident from a doublet at δ 5.34 integrating one proton. A one proton multiplet was found at δ 3.52. The position and multiplicity of which was indicative of H-6 of the steroidal nucleus. The spectrum further revealed signals at δ 0.67 and δ 1.00 (3H each) assignable to two tertiary methyl groups at C-18 and C-19, respectively. The ¹H NMR spectrum showed two doublets centered at δ 0.83 ($J = 7.2$ Hz) and at δ 0.81 ($J = 7.2$ Hz) which could be attributed to two methyl groups at C-25. The doublet at δ 0.91 ($J = 6.4$ Hz) was demonstrative of a methyl group at C-20. On the other hand, the triplet of three-proton intensity at δ 0.85 could be assigned to the primary methyl group attached to C-28.

The above spectral features (Table-3) are in close agreement to those observed for β -sitosterol (Dhaka Univ. J. Sci. 59(2): 179-180, 2011 (July)). On this basis, the identity of SJ-92 was confirmed as β -sitosterol. The identity of SJ-92 was further confirmed by CO-TLC with authentic sample, previously isolated in Phytochemical Research Laboratory.

3.1.3. Characterization of SJ-184 as 3-nor-2,4-Secofriedelan-4-oxo-2-oic Acid Derivative

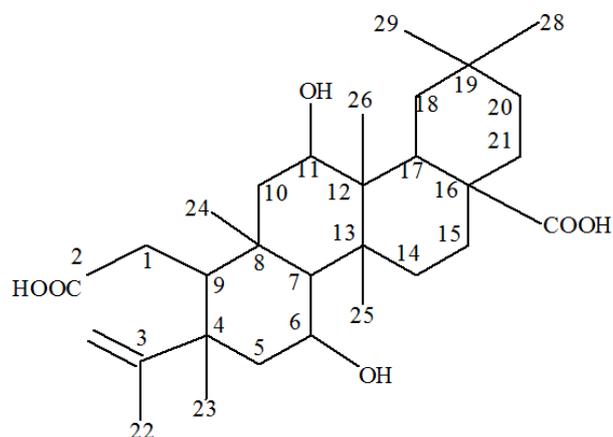


Figure 5. Compound SJ-184 (3-nor-2,4-Secofriedelan-4-oxo-2-oic Acid Derivative)

Compound SJ-184 was obtained (Fig-5) from the VLC fraction 16 (20% methanol in ethyl acetate) of crude extract as white crystals. It was evident as a purple spot on TLC (Silica gel PF₂₅₄) when the developed plate was sprayed with vanillin-sulfuric acid followed by heating at 110°C for 5-10 minutes. It was found to be soluble in petroleum ether, hexane, ethyl acetate and chloroform.

The ¹H NMR spectrum (500 MHz, CDCl₃) of SJ-184 (Figure-6 & table-3) revealed signal for multiplet of proton intensity at δ 2.27 typical for H-1. Seven singlet signals at 2.16ppm, 0.73ppm, 0.80ppm, 0.89ppm, 0.98ppm, 1.23ppm and 0.94ppm, integrating for three protons assignable to seven tertiary methyl groups at C-22, C-23, C-24, C-25, C-26, C-28 and C-29 respectively.

Three proton singlet in the more deshielded region ($\delta = 2.16$ ppm) for methyl group at C-22. This high chemical shift is due to the attachment to ketone group.

From this above spectral features it is assumed that the compound have seven methyl protons, one of which is attached to a ketone group. So, it is proposed that the compound SJ-184 may be 3-nor-2,3-secofriedelan derivative.

Table 3. ¹H NMR spectral data of SJ-184 as 3-nor-2,3-secofriedelan derivative (proposed) (J. Agric. Food Chem. 2006, 54, 3566–3571)

Protons	Chemical shift
H-1	2.27 (1H, m)
H-22	2.16 (3H, s)
H-23	0.73 (3H, s)
H-24	0.80 (3H, s)
H-25	0.89 (3H, s)
H-26	0.98 (3H, s)
H-28	1.23 (3H, s)
H-29	0.94 (3H, s)

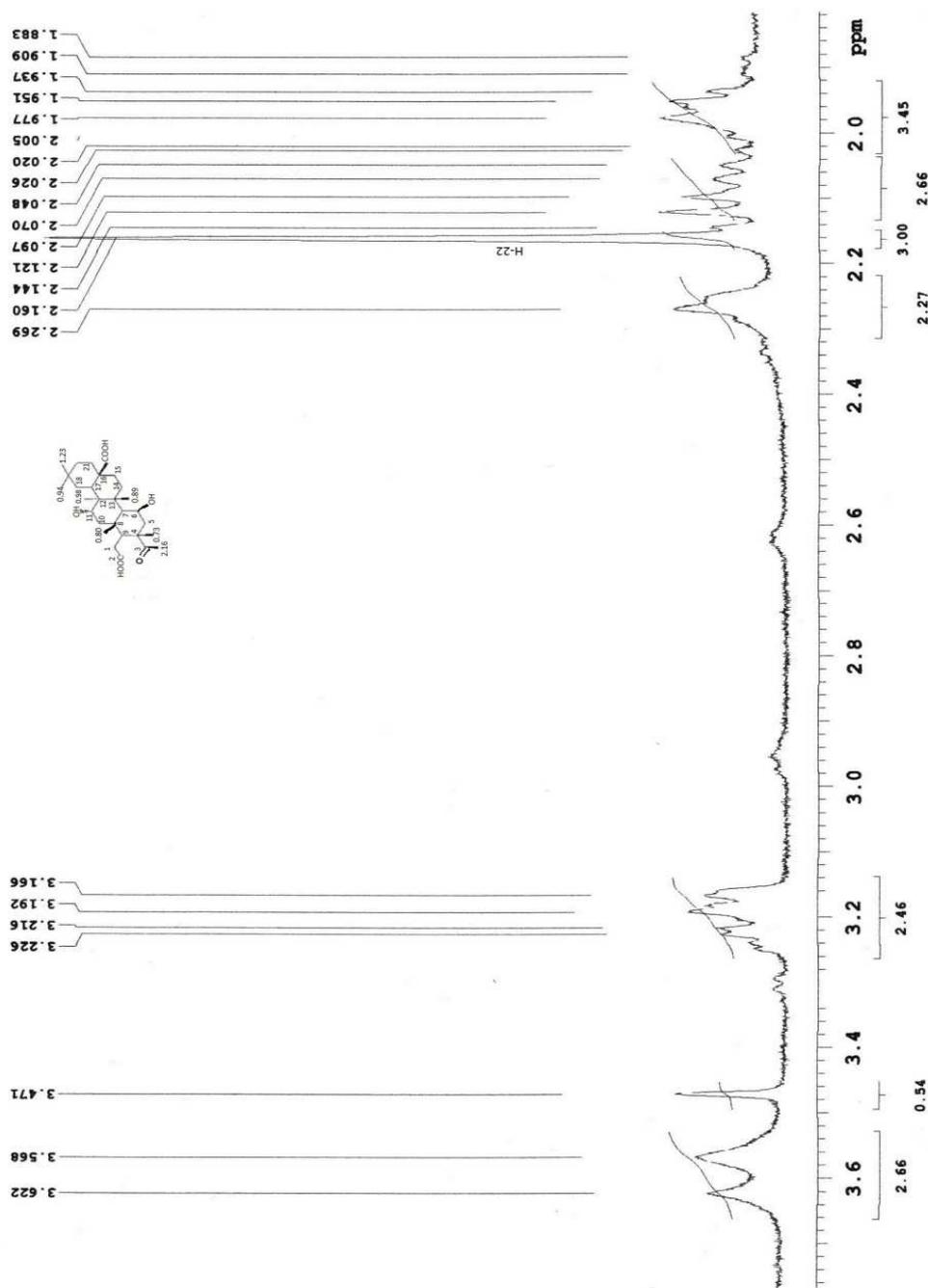


Figure 6. ¹H NMR spectrum of SJ-184 in CDCl₃

3.2. Brine Shrimp Lethality Assay of the Purified Crude Extract of *S. Jambos*

In the present bioactivity study all the crude extracts, hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of methanolic extract showed positive results indicating that the test samples are biologically active. Each of the test samples showed different mortality rates at different concentrations. Plotting of log of concentration versus percent mortality for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC₅₀, the concentration at which 50%

mortality of brine shrimp nauplii occurred) was determined for the samples.

The methanolic extract of bark and the different partitionate of the methanolic extract i.e. hexane soluble partitionate (HSF), carbon tetrachloride soluble partitionate (CTCSF), chloroform soluble partitionate (CSF) and aqueous soluble partitionate (AQSF) of *S.jambos* were subjected to brine shrimp lethality bioassay following the procedure of Meyer *et al.*, (1982). The lethality of the extractives to brine shrimp was determined and the results are given in Table-4.

The lethal concentration LC₅₀ of the test samples after 24 hr. was obtained by a plot of percentage of the shrimps died

against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis.

Vincristine sulfate (VS) was used as positive control and the LC₅₀ was found 0.451 µg/ml for VS. Compared with the negative control VS (positive control) gave significant mortality and the LC₅₀ values of the different extractives

were compared to this positive control.

The LC₅₀ values of HSF, CTCSF, CLSF and MESSJ were found to be 257.596µg/ml, 13.616µg/ml, 64.943 µg/ml, and 6.97µg/ml respectively. MESF and CTCSF showed significant lethality whereas CLSF showed moderate activity and HSF showed least activity.

Table 4. LC₅₀ values of the test samples of *S.jambos*

Test samples	Regression line	R ²	LC ₅₀ (µg/ml)
VS	y = 30.799x + 60.64	0.9729	0.451
MESF	y = 34.629x+20.803	0.9497	6.97
HSF	y = 19.485x+3.3579	0.972	247.596
CTCSF	y = 39.689x + 4.9914	0.9463	13.616
CLSF	y = 35.233x-14.95	0.8681	69.734
AQSF	y = 35.233x + 11.05	0.982	12.75

VS = Vincristine sulphate, MESF = Methanol soluble fraction, HSF = Hexane soluble fraction, CTCSF = Carbon tetrachloride soluble fraction, CLSF = Chloroform soluble fraction

4. Conclusion

Different partitionates of the methanolic extract of the stem bark of *S. jambos* were investigated for isolation of the potent secondary metabolites of this plant. Successive chromatographic separation and purification of the VLC partitionates of the crude methanolic extract yielded a total of three compounds. The structure of these compounds were elucidated as β-sitosterol, friedelin and 3-nor-2,3-secofriedelin derivative (proposed).The chloroform soluble fraction and the carbon tetrachloride soluble fraction of the stem bark of *S. jambos* exhibited mild to moderate antimicrobial activity. Methanolic extract of stem barks and the chloroform soluble fraction of this plant showed significant free radical scavenging activity.

In addition, the crude methanolic extract of stem barks, the chloroform partitionate, and carbon tetrachloride of the crude extract of the plant gave better cytotoxic effect.

In conclusion, considering the potential bioactivity, this plant can be studied extensively to find out their unexplored efficacy and to rationalize their use as traditional medicine. Further investigation will lead to isolation, purification and characterization of new molecules from this plant.

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