STRUCTURAL ELUCIDATION OF PURE ORGANIC COMPOUND ISOLATED FROM THE BARK OF *MYRICA NAGI* THUNB.

Tin Myo Khaing[1], Khaing Khaing Kyu[2], Thida Win[3]

Abstract

In this research work, one of the Myanmar medicinal plants, *Myrica nagi* Thunb. (Local name : Kat pha la) was selected for the chemical analysis. Preliminary detection of phytochemical compounds present in the bark of Kat pha la were carried out according to the test tube method. The percent composition of elements of the dry powder from the bark of Kat pha la was determined by using WDXRF spectrum. The antimicrobial activities of the crude extracts and the pure organic compound (TMK-1) were tested by agar well diffusion method using six organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*). The pure organic compound (TMK-1) was isolated from the bark of Kat pha la by thin layer and column chromatographic separation techniques. Melting point and phytochemical test of pure organic compound (TMK-1) were recorded. The molecular formula was determined by using FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, DQF-COSY, HMQC, HMBC spectroscopy and Mass spectrometry. In addition, the complete structure of terpene derivative compound (TMK-1) was elucidated by applying 1D and 2D NMR spectroscopic techniques as well as EI-Mass spectrometry. Moreover the conformational structure of isolated compound (TMK-1) was examined by using ^{1}H NMR spectrum, NOESY spectrum and the model study.

Keywords: *Myrica nagi* Thunb., Kat pha la, WDXRF, Chromatographic methods

Introduction

Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhance activity and reduced toxicity. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. Where the active molecule may be obtained from the cultivation of plant material. The scientific study of the traditional medicines, derivation of drugs through bio-prospecting and systematic conservation of the concerned medicinal plants are thus of great importance (Fransworth and Soejarto, 1985).

In this research work, One Myanmar traditional indigenous plant, Kat pha la belongs to *Myrica nagi* Thunb. was chemically analyzed for new source of compound in this field (Figure 1). The bark of Kat pha la is used as an antirheumatic, an antiseptic, aromatic, an astringent, carminative, ophthalmic and a stimulant in indigenous medicine. It is used as a remedy for various body disorders such as liver diseases, fever, asthma, anaemia, chronic dysentery, ulcer and inflammation (Rastogi and Mehrotra, 1985).

Firstly, pre-phytochemical screening and antimicrobial activities of crude extract of *Myrica nagi* Thunb. were carried out. As an experimental work, a pure organic compound (TMK-1) could be isolated from the bark of Kat pha la by using thin layer and column chromatographic methods.

Its structure could be elucidated by using advanced spectroscopic methods, such as FT IR, ^{1}HNMR (500 MHz), ^{13}CNMR (125 MHz), DEPT, DQF-COSY, HMQC and HMBC spectroscopy and EI-MS spectrometry. The conformational analysis of pure organic compound

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(TMK-1) was determined by using splitting pattern and coupling constants of proton in ^{1}H NMR spectrum, NOESY spectroscopy and modern study.

Figure 1 Tree, leaves, flowers, and bark of Kat pha la

Materials and Methods

Sample Collection

The bark of Kat pha la for experiment was collected from Pyinsa Village, Pyin Oo Lwin Township, Mandalay Region, Myanmar. The samples were cut into small pieces and allowed to air dry. Then, the dried species were stored in a well-stoppered bottle and used throughout the experiment.

Preliminary Phytochemical Test on the Sample

Preliminary detection of phytochemical compounds present in bark of *Myrica nagi* Thunb. was carried out according to the test tube methods (Harborne,1993).

Elemental content of Sample

The elements present in dried powdered sample were determined by WDXRF (Wave Dispersive X-ray Fluorescence) spectrometer (Rigaku supermini-200).

Examination of Antimicrobial Activity

The antimicrobial activity of crude extracts of sample was examined using agar well diffusion method.

Extraction and Isolation of the Pure Organic Compounds

Air dried sample (1000 g) was percolated with 2500 mL of 95 % ethanol for about one month. Percolated solution was filtered off and the filtrate was evaporated at room temperature. The ethanol crude extract was re-extracted with ethylacetate (300 mL) and the filtrate was also evaporated ethylacetate extract (2.02 g) was obtained. The crude extract was separated by column chromatography applying SiO_2 (70-230 mesh) as an adsorbent with n-hexane and ethylacetate solvent system. Totally 158 fractions were obtained. Each fraction was checked by TLC with suitable solvent system. The fractions with the same R_f value were combined to give five combined fractions. The fraction D shows nearly one spot on TLC plate and it was the major constituent. So, it was further re-chromatographed by using the same procedure as described above. All fractions were again checked by TLC and UV detector. Four combined fractions were obtained and evaporated at room temperature. The fraction IV shows only one spot on TLC plate and under UV detector. Finally, 14.02 mg of the pure organic compound (amorphous form) was obtained. This compound was sent to Japan for measurement of invaluable spectroscopic data.

Determination of Melting Point and Phytochemical Screening of the Pure Organic Compound (TMK-1)

A few white amorphous form of pure organic compound (TMK-1) were inserted into the capillary tube and the melting point was determined by using the melting point apparatus. The phytochemical test was carried out to identify the class of pure compound (TMK-1). A mixture of few drops of concentrated sulphuric acid, 1 mL of acetic anhydride and 2.5 mL of chloroform was added to ethanol extract of pure organic compound (TMK-1).

Determination of Antimicrobial Activities of the Pure Organic Compound (TMK-1)

Antimicrobial activities of pure compound (TMK-1) were tested by agar well diffusion method at Pharmaceutical Research Department (PRD).

Results and Discussion

Preliminary Phytochemical Examination of Kat pha la

The results of preliminary phytochemical test of the bark of Kat pha la are tabulated in Table 1.

Table 1 Preliminary Phytochemical Examination of Kat pha la

No	Constituent	Reagent used	Observation	Remark
1	Alkaloid	Mayer's reagent	White ppt	+
2	Flavonoid	Conc: HCl, Mg coil	Pink colour solution	+
3	Terpenoid	$CHCl_3$,Conc:H_2SO_4,$(CH_3CO)_2O$	Brown red solution	+
4	Steroid	$(CH_3CO)_2O$, Conc:H_2SO_4	Green colour solution	+
5	Glycoside	NaOH	Yellow colour solution	+
6	Reducing sugar	Benedict's solution	Brick red ppt	+
7	Tannin	1% $FeCl_3$, Dil:H_2SO_4	Yellowish brown ppt	+
8	Phenolic	10% $FeCl_3$	Green colour solution	+
9	Saponin	Distilled water	Frothing	+

(+) = presence of the constituents (-) = absence of the constituents

According to the preliminary phytochemical test, all varieties of phytochemical constituents were present in the bark of Kat pha la.

Elemental Analysis of Kat pha la by WDXRF

From the WDXRF spectrum, the percent composition of elements of the dried powder from the bark of Kat pha la could be determined. By WDXRF spectral data (Table 2), the total number of elements is 12 elements in the bark of Kat pha la. Among them, calcium, sodium and potassium were found as major elements. Iron, silicon and chlorine were also found as minor elements. Magnesium and other elements were trace elements. The order of decreasing concentration of the elements were Ca > Na > K > Fe > Si > Cl > Mg > Mn > S > Al > P> Sr.

Table 2 Relative Abundance of the Dried Powder from Bark of Kat pha la

No	Element	Relative abundance (%)
1	Na	18.3
2	Mg	1.33
3	Al	0.739
4	Si	4.71
5	P	0.45
6	S	1.25
7	Cl	2.11
8	K	9.29
9	Ca	24.6
10	Mn	1.28
11	Fe	5.53
12	Sr	0.101

Antimicrobial Activities of Bark of Kat pha la

The results of antimicrobial activity of the sample are shown in Table 3.

Table 3 Antimicrobial activities of one Myanmar indigenous Medicinal Plant (Kat pha la)

Sample	Solvents	Inhibition Zone Diameters (mm) of Various Crude Extracts Against Different Microorganisms					
		B.Subtilis	***S.aureus***	***P.aeruginosa***	***B.pumilus***	***C.albicans***	***E.coli***
Kat pha la (bark)	n-hexane	–	–	–	–	–	–
	$CHCl_3$	14 (+)	15 (+)	13 (+)	–	–	–
	Acetone	15 (++)	20 (+++)	14 (+)	19 (++)	20 (+++)	15 (++)
	EtOAc	23 (+++)	25 (+++)	25 (+++)	25 (+++)	28 (+++)	20 (+++)
	EtOH	18 (++)	19 (++)	14 (+)	20 (+++)	20 (+++)	20 (+++)

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

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

The ethylacetate extract of bark of Kat pha la responded high activities on all tested organisms. In contrast, n-hexane extract did not show activity on all tested organisms. Ethanol extract showed high activities on *Bacillus pumilus, Candida albicans* and *Escherichia coli* and medium activities on *Bacillus subtilis* and *Staphylococcus aureus* and low activity on *Pseudomonas aeruginosa*. Acetone extract showed high activities on *Staphylococcus aureus* and *Candida albicans* and medium activities on *Bacillus subtilis, Bacillus pumilus* and *Escherichia coli* and low activity on *Pseudomonas aeruginosa*. Moreover, chloroform extract showed low activities on *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* and did not show activity on *Bacillus pumilus, Candida albicans* and *Escherichia coli.* So, pure organic

compound (TMK-1) was isolated from the ethylacetate extract of bark of Kat pha la applying separation techniques.

Antimicrobial Activities of the Pure Compound (TMK-1)

The results of the antimicrobial test relevant to different types of organisms are tabulated in Table 4.

Table 4 Antimicrobial Activities of Pure Organic Compound (TMK-1)

No	Types of Organisms	Inhibition zone diameter (mm)
1.	*Bacillus subtilis*	11 (+)
2.	*Staphylococcus aureus*	12 (+)
3.	*Pseudomonas aeruginosa*	13 (+)
4.	*Bacillus pumilus*	11 (+)
5.	*Candida albicans*	13 (+)
6.	*Escherichia coli*	11 (+)

In accordance with Table 3, the pure organic compound (TMK-1) responds low activity on all tested microorganisms.

Determination of Melting Point and Phytochemical Testing for the Pure Organic Compound (TMK-1)

The melting point was found to be 239-241°C. Pure compound (TMK-1) showed positive test for terpenoid. So it may be terpenoid compound.

Molecular Formula Determination of the Pure Organic Compound (TMK-1)

Molecular formula of pure organic compound (TMK-1) could be determined by using FT-IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125MHz), DEPT, DQF-COSY, HMQC, HMBC and EI-mass spectral data. (Crews *et al.,* 1998; Morrison and Boyd, 2000; Silverstein and Webster, 1998)

Infrared Spectrum of the Pure Organic Compound (TMK-1)

The FT IR spectrum of isolated pure organic compound (TMK-1) was measured at the Department of Chemistry, University of Mandalay. It is described in Figure 2 a.

According to FT IR spectral data, compound consists of –OH stretching vibration of alcohol group, –CH stretching vibration of alkenic group, unsymmetrical and symmetrical –CH stretching vibration of sp^3 hydrocarbons, –C=C stretching vibration of alkenic group,–CH in plane bending vibration of allylic hydrocarbon, -CH out of plane bending vibration of gem dimethyl group, –C-C-O stretching vibration of alcohol group and –CH out of plane bending vibration of trans or E and cis or Z alkenic groups respectively (Table 5).

Table 5 FT IR Assignments of Pure Organic Compound (TMK-1)

Absorption band (cm^{-1})	Assignments
3448	–OH stretching vibration
3066	–CH stretching vibration of alkenic group
2931, 2877	sp^3CH stretching vibration of asymmetric and symmetric hydrocarbon
1643	–C=C stretching vibration of alkenic group
1454	–CH in plane bending vibration of allylic group
1373	–CH out of plane bending vibration of gem-dimethyl group
1191, 1091	–C–C–O stretching vibration of alcohol group
929, 798	–CH out of plane bending vibration of trans or E and cis or Z alkenic group

^{1}H NMR Spectrum of Pure Organic Compound (TMK-1)

The ^{1}H NMR spectrum (500 MHz) is described in Figure 2 b. In accordance with this spectrum, the number of protons could be calculated as (50) in pure organic compound (TMK-1).

^{13}C NMR and DEPT Spectral Data of the Pure Organic Compound (TMK-1)

The ^{13}C NMR (125 MHz) spectrum (Figure 2 c) indicated the number of carbons to be (30) in the pure organic compound (TMK-1). Also DEPT spectrum (Figure 2 d) gave information of variety of hydrocarbons.

According to ^{1}H NMR (Figure 2 b), ^{13}C NMR (Figure 2 c), DEPT (Figure 2 d) and HMQC (Figure 2 e) spectral data, the partial molecular formula of pure compound (TMK-1) is $C_{30}H_{49}$ and partial molecular mass is 409 Da.

But the molecular mass of pure organic compound (TMK-1) is 426 Da according to EI-MS spectrum (Figure 2 f). So, the remaining molecular mass is 17 Da. The FT IR spectrum of the isolated compound (Figure 2 a) shows that this compound should contain one hydroxyl group. It is confirmed by FT IR spectrum in which the hydroxyl group is found to be at (3448 cm^{-1}). Due to the remaining molecular mass 17 Da and the chemical shifts of carbons and protons in their respective spectra, the pure compound should contain one hydroxyl group. So, the molecular formula of the pure isolated organic compound (TMK-1) would be $C_{30}H_{50}O$.

$$\text{Hydrogen Deficiency Index} = C - \frac{H}{2} + 1$$

$$= 30 - \frac{50}{2} + 1 = 6$$

Its hydrogen deficiency index is 6. The calculated molecular mass of the isolated pure organic compound is in agreement with the measured molecular mass of that compound (m/z = 426 Da).

Confirmation of Molecular Formula of Compound (TMK-1)

Molecular formula of compound (TMK-1) could be confirmed by DEPT spectrum (Figure 2 d) and FT IR spectrum (Figure 2 a).

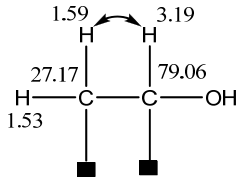
Table 6 Results Given by DEPT and FT IR Spectral Data of Compound (TMK-1)

Assignments	No. of Carbon	No. of Proton	No. of Oxygen
DEPT Spectrum			
-Six sp^3 quaternary carbons	6	-	-
-Four sp^3 methine carbons	4	4	-
-Ten sp^3 methylene carbons	10	20	-
-Eight sp^3 methyl carbons	8	24	-
-One sp^2 methine carbon	1	1	-
-One sp^2 quaternary carbon	1	-	-
FT IR Spectrum			
-One –OH group	-	1	1
Complete Molecular formula	C_{30}	H_{50}	O

Structure Elucidation of Pure Organic Compound (TMK-1)

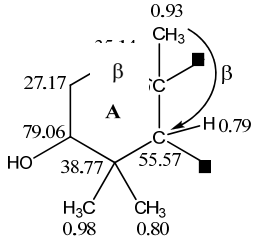
The structure of pure organic compound (TMK-1) could be elucidated by applying ^{1}HNMR, DEPT, HMQC, DQF-COSY and HMBC respectively.

In DQF-COSY spectrum (Figure 2 g), the correlation between sp^3 methylene protons (δ 1.53 and 1.59 ppm) and sp^3 methine proton (δ 3.19 ppm) gives the following fragment (1). In HMQC spectrum (Figure 2 e), the sp^3 methine proton (δ 3.19 ppm) directly attaches to the carbon (δ 79.06 ppm). The chemical shifts of these proton and carbon show that this carbon should be carbinol carbon.



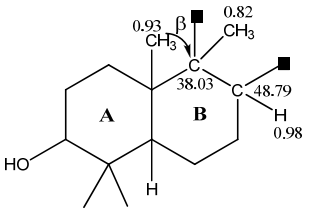
Fragment (1)

In HMBC spectrum (Figure 2 h), the β ^{1}H- ^{13}C long range signal of sp^3 methyl protons (δ 0.93 ppm) with sp^3 methine carbon (δ 55.57 ppm) gives the fragment (2) including the six- member ring (A).



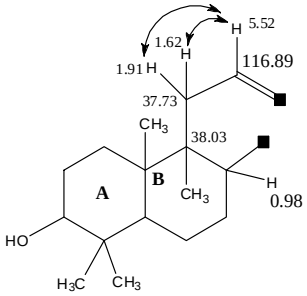
Fragment (2)

In HMBC spectrum (Figure 2 h) the β ^{1}H-^{13}C long range coupling between the sp^3 methyl protons (δ 0.93 ppm) and sp^3 quaternary carbon (δ 38.03 ppm) gives the fragment (3) including six member ring (B).



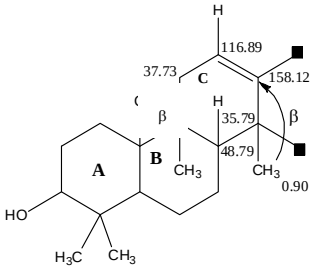
Fragment (3)

The correlations between the sp^3 methylene protons at δ (1.62 and 1.91 ppm) and sp^2 methine proton at (δ 5.52 ppm) in DQF- COSY spectrum (Figure 2 g) give rise to the fragment (4).



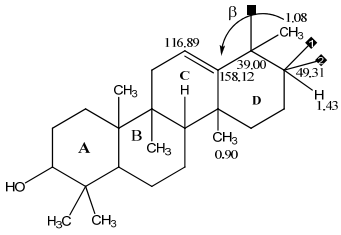
Fragment (4)

The β ^1H-^{13}C long range correlation of sp^3 methyl protons at (δ 0.90 ppm) and sp^2 quaternary carbon at (δ 158.12 ppm) in HMBC spectrum (Figure 2 h) produces the fragment (5) including six member ring (C).



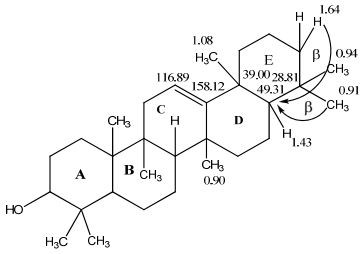
Fragment (5)

The β ^1H-^{13}C long range correlation of sp^3 methyl protons at (δ 1.08 ppm) and sp^2 quaternary carbon at (δ 158.12 ppm) in HMBC spectrum (Figure 2 h) gives the following fragment (6) including ring D.



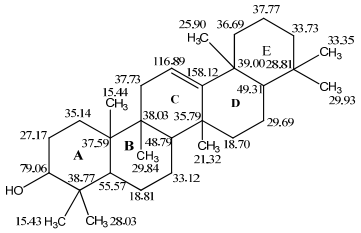
Fragment (6)

Furthermore, the β ^1H- ^{13}C long range signal of sp^3 methyl protons (δ 0.91 ppm) with this sp^3 methine carbon (δ 49.31 ppm) in HMBC spectrum (Figure 2 h) gives the fragment (7) closing the six member ring (E) as follows. It could be confirmed by the correlations between sp^3 methylene proton at (δ 1.64 ppm) with sp^3 methine carbon at (δ 49.31 ppm) in HMBC spectrum (Figure 2 h).



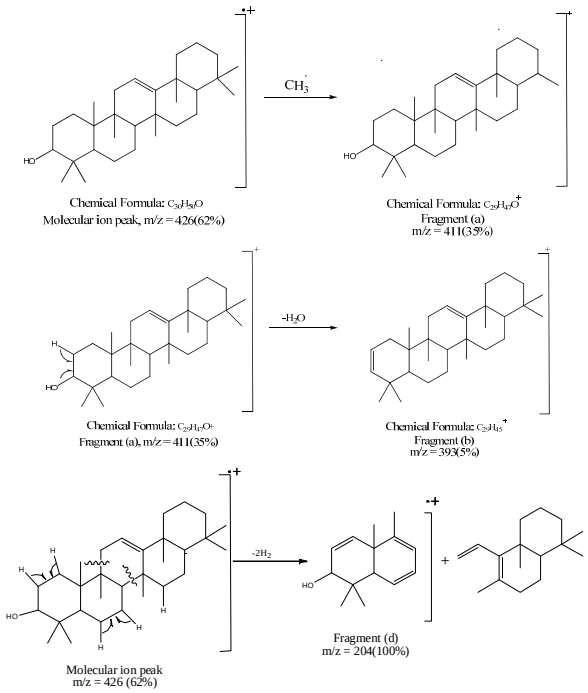
Fragment (7)

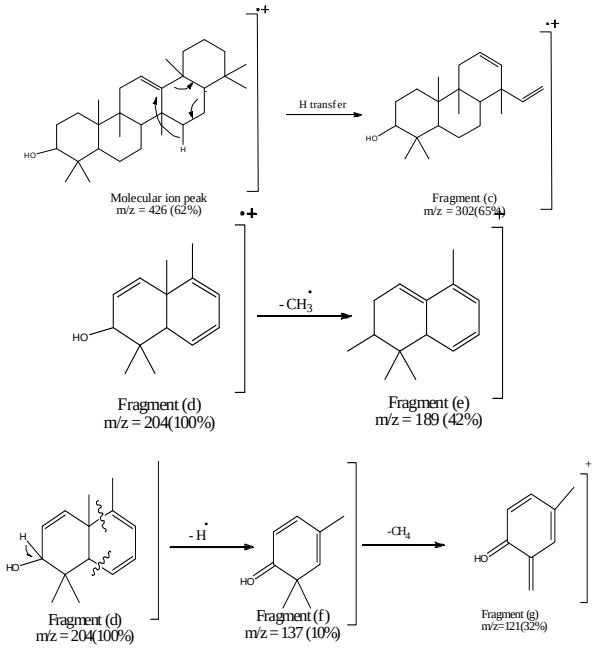
The complete planar molecular structure of the isolated pure organic compound (TMK-1) could be expressed as follows and the name of the isolated compound is (3S, 4aR, 6aR, 6bS, 8aS, 12aS, 14aS, 14bS)- 4, 4, 6b, 9, 9, 12a, 14a, 14b-octamethyl-1, 2, 3, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b- icosahydropicen- 3- ol.



Mass Fragmentation Behaviour of the Pure Organic Compound (TMK-1) (Porter, 1971)

The structure of the compound (TMK-1) could be confirmed by EI-MS fragmentation behavior. The proposed mechanisms of the formation of fragment ion peaks formed from compound (TMK-1) (Figure 2 f) could be described as follows.



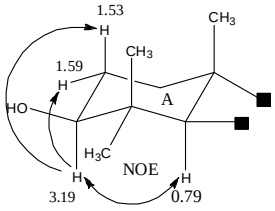


Conformational Analysis of the Pure Organic Compound (TMK-1)

The conformational analysis of pure organic compound (TMK-1) was assigned on the basis of the splitting patterns and the coupling constants of the protons in ^{1}H NMR spectrum, NOESY spectral data and the model study.

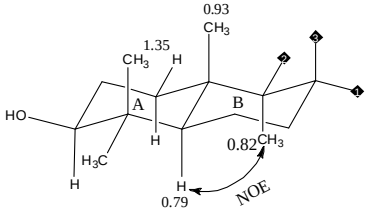
The splitting pattern of the carbinol proton at δ 3.19 ppm is double doublet and its J values are 13.5 and 5.0 Hz respectively. It is coupled with the axial proton (δ1.53 ppm) and the equatorial proton (δ 1.59 ppm). So, its coupling constant values show that this carbinol proton is at axial position (below the plane) giving the following chair like conformer of ring (A).

3.19 ppm,
dd, J = 13.5,5.0 Hz

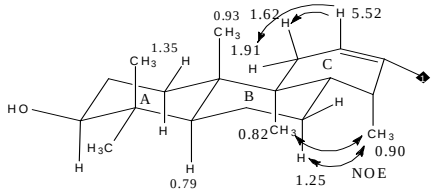


It is confirmed by the medium NOE with the axial Junction methine proton (δ 0.79 ppm) in NOESY spectrum (Figure 2 i) show that the junction methine proton is below the plane showing the chair conformer of ring (A).

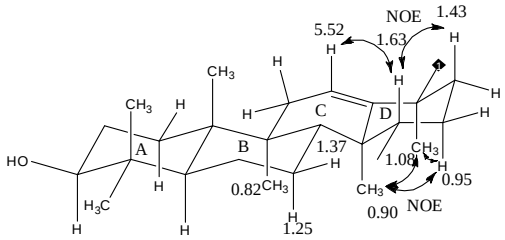
The chair conformer of ring (B) was assigned by the model study. It was confirmed by the medium NOE correlation between axial junction methine proton (ring A and B) (δ 0.79 ppm) and the other junction methyl protons (ring B and **C)** (δ 0.82 ppm) in NOESY spectrum (Figure 2 i) showing the chair conformer of ring (B).



The chair conformer of ring (B) and the boat like conformer of ring (C) were assigned by the model study. The splitting pattern of the sp^2 methine proton at (δ 5.52 ppm) is double doublet and its J values are 10.0 and 5.0 Hz respectively. It is coupled with the axial proton (δ 1.62 ppm) and the equatorial proton (δ 1.91 ppm). So, its coupling constant values show that this sp^2 methine proton is at axial position (above the plane) giving the following boat conformer of ring (C).

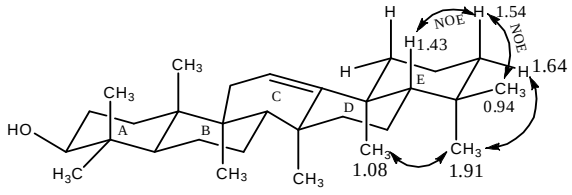


It was confirmed by the medium NOE correlation between the junction methyl protons (rings B and C) (δ 0.82 ppm) and (rings C and D) (δ 0.90 ppm) which is correlated with methylene proton (δ 1.25 ppm) (small NOE) in NOESY spectrum (Figure 2 i) showing all these protons are below the plane. The chair conformer of ring (D) was assigned by the modern study. The medium NOE between the junction methyl protons (ring C and D) (ring D and E) (δ 0.90 ppm and 1.08 ppm) and one of methylene protons (δ 0.95 ppm) in NOESY spectrum (Figure 2 i) show that all these protons are under the plane of their respective ring.



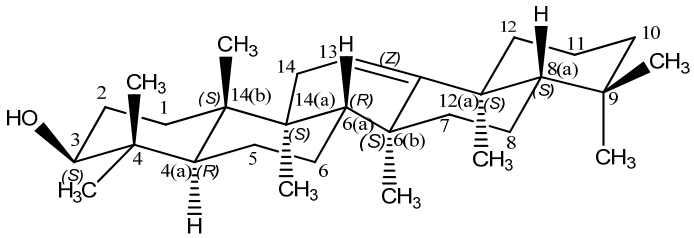
It was confirmed by the cross peak between the sp^2 methine proton in ring C (δ 5.52 ppm) and axial methylene proton (δ 1.63 ppm) which is correlated with junction methine proton (δ 1.43 ppm) (by medium NOE) in NOESY spectrum (Figure 2 i) showing all these protons are upper the plane.

The chair conformer of ring (E) was assigned by the modern study. It is confirmed by the junction methine proton (ring D and E) (δ 1.43 ppm) is medium NOE with axial methylene proton (δ 1.54 ppm) which is correlated with equatorial methyl protons (δ 0.94 ppm) showing all these protons are upper the plane in NOESY spectrum (Figure 2 i).



In addition, the junction methyl protons (ring D and E) (δ 1.08ppm) is medium NOE with axial methyl protons (δ 0.91 ppm) which is correlated with equatorial methylene proton (δ 1.64 ppm) (by small NOE) showing all these protons are under the plane in NOESY spectrum (Figure 2 i).

The complete conformational structure of the isolated pure organic compound (TMK-1) and its absolute configuration are shown below.



The relative conformation of eight chiral carbons could assigned as C_3 (S), $C_{4(a)}$ (R), $C_{6(a)}$(R), $C_{6(b)}$ (S), $C_{8(a)}$ (S), $C_{12(a)}$ (S), $C_{14(a)}$ (S), $C_{14(b)}$ (S) respectively.

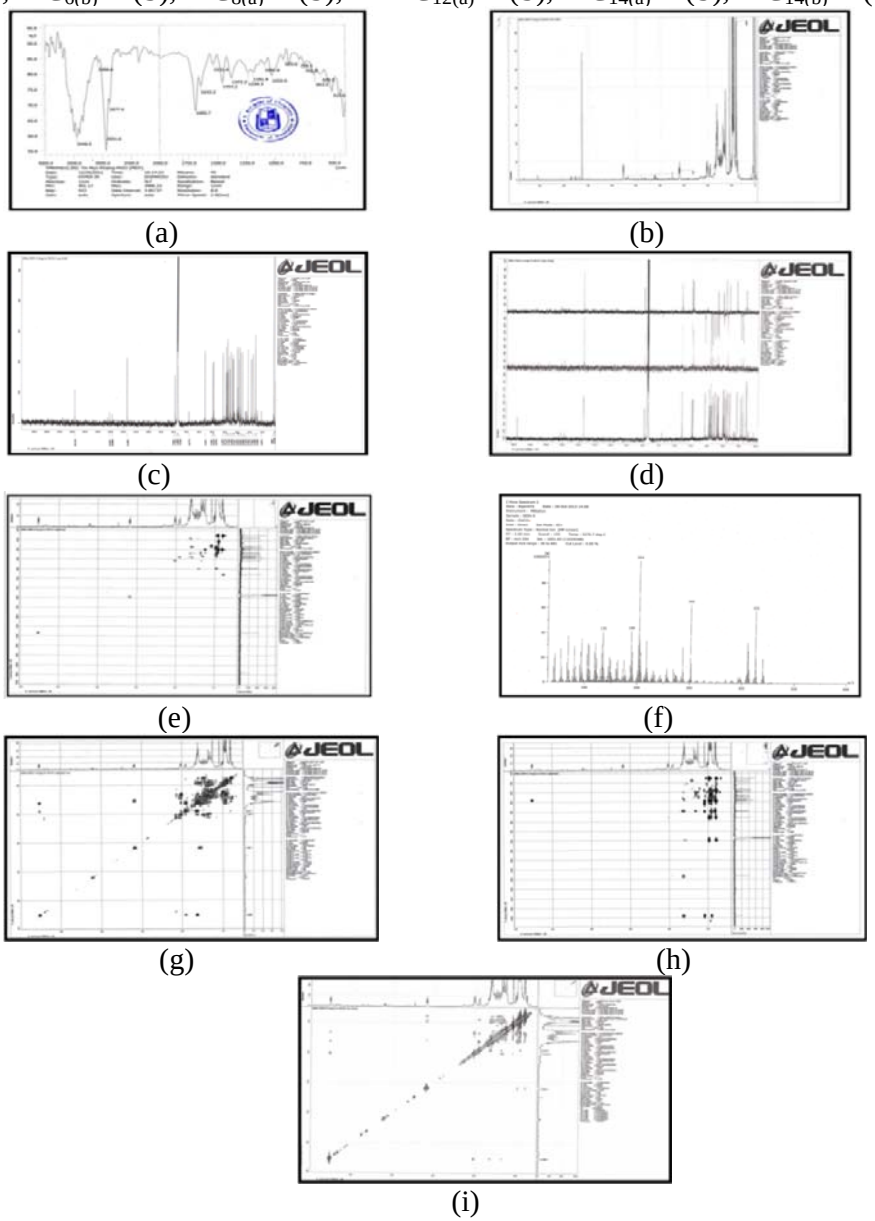


Figure 2 (a) FT IR, (b) ^{1}HNMR, (c) ^{13}C NMR, (d) DEPT, (e) HMQC,
(f) EI Mass, (g) DQF-COSY and (h) HMBC
(i) NOESY-Spectrums of the Pure Organic Compound (TMK-1)

Conclusion

In this research work the bark of *Myrica nagi* Thunb. was selected for the chemical analysis. The bark of Kat pha la contains alkaloid, flavonoid, terpenoid, reducing sugar, glycoside, tannin, phenolic and saponin compounds. The element compositions from the bark of Kat pha la were detected by using WDXRF spectrum. From the WDXRF spectral data, the total number of 12 elements was detected in the bark of Kat pha la. Among them**,** Ca, Na and K were found as major elements. Fe, Si and Cl were also found as minor elements. Mg and other elements were trace elements.

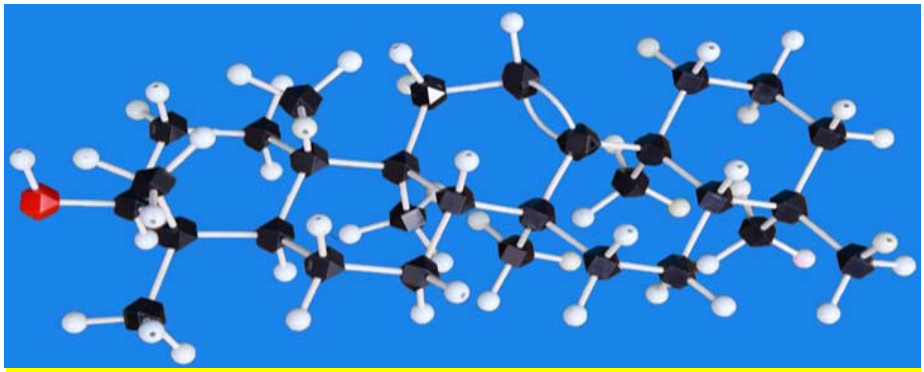
In addition, antimicrobial activities of the crude extracts and the pure organic compound (TMK-1) were tested by agar well diffusion method using six organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*). Particularly, the ethylacetate extract of bark of Kat pha la showed high activities on all tested organisms.

Moreover, the pure organic compound (TMK-1) could be isolated by thin layer and column chromatographic separation techniques. The pure organic compound (TMK-1) was obtained as pale white amorphous compound. The yield percent of the isolated compound is 0.694% based upon the EtOAc crude extract. Phytochemical test for pure compound (TMK-1) was done and it showed the positive test for terpenoid. The melting point of this compound was determined and found to be 239-241 °C. This pure compound (TMK-1) was to possess low activity on all tested organism.

Furthermore, the FT IR spectrum indicated the presence of hydroxyl group at 3448 cm^{-1}. The mass spectroscopy displayed (M^+) at m/z 426. Its formula is $C_{30}H_{50}O$ and hydrogen deficiency index is 6. The ^1H NMR spectrum reflected eight methyl groups, a hydroxyl methylene group and one olefinic proton. Finally, the complete structure analysis of the isolated pure compound (TMK-1) could be assigned by NOESY and ^1H NMR spectra. The conformational structure of pure compound (TMK-1) contains the three chair like conformer of ring A, B and D and boat conformer of ring C. The name of the pure organic compound (TMK-1) is (3S, 4aR, 6aR, 6bS, 8aS, 12aS, 14aS, 14bS)- 4, 4, 6b, 9, 9, 12a, 14a, 14b-octamethyl-1, 2, 3, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b- icosahydropicen- 3- ol.

In this plant sample, valuable phytochemical compounds were found and the ethylacetate extract of this plant sample give high activity on all selected organisms. Therefore, this plant sample is suitable for medicinal purposes, further studies will be required.

CONFORMATIONNAL STRUCTURE OF PURE ORGANIC COMPOUND (TMK-1)



(3S, 4aR, 6aR, 6bS, 8aS, 12aS, 14aS, 14bS)- 4, 4, 6b, 9, 9, 12a, 14a, 14b-octamethyl-1, 2, 3, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b- icosahydropicen- 3- ol

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