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# ISOLATION OF ALKALOIDS FROM *Alseodaphne pendulifolia* Gamb. (LAURACEAE) AND THEIR BIOLOGICAL ACTIVITIES



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MUHAMMAD HAFIZ HUSNA BIN HASNAN

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FACULTY OF SCIENCE AND MATHEMATICS  
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2014



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## INSTITUTE OF GRADUATE STUDIES

### DECLARATION OF ORIGINAL WORK

This declaration is made on the .....8<sup>th</sup>.....day of AUGUST 2014.

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I, MUHAMMAD HAFIZ HUWAIB BIN HASNAN, M20102001203, F2MFT (PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled ISOLATION OF ALKALOIDS FROM ALSEODAPHNE PENDULIFOLIA GAMB. (LAURACEAE) AND THEIR BIOLOGICAL ACTIVITIES is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

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#### ii. Supervisor's Declaration:

I Assoc. PROF. DR. MOHD AZLAN NAFIAH (SUPERVISOR'S NAME) hereby certifies that the work entitled ISOLATION OF ALKALOIDS FROM ALSEODAPHNE PENDULIFOLIA GAMB. (LAURACEAE) AND THEIR BIOLOGICAL ACTIVITIES (TITLE) was prepared by the above named student, and was submitted to the Institute of Graduate Studies as a \* partial/full fulfillment for the conferment of MASTER OF SCIENCE (NATURAL PRODUCTS) (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

Date

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## ABSTRACT

The purpose of this study is to extract, isolate and elucidate the structure of alkaloid constituents from barks of *Alseodaphne pendulifolia* Gamb. (Lauraceae) and to determine the biological activities (antiplasmodial, cytotoxicity, antibacterial and antioxidant) against the isolated alkaloids. The samples were collected from Sg. Temau Forest Reserve, Kuala Lipis, Pahang and Sg. Tekam Forest Reserve, Jerantut, Pahang, Malaysia. The isolation and purification of alkaloids were done using various chromatographic techniques including chromatotron, column, preparative thin layer and thin layer chromatography. The structure elucidations of the isolated alkaloids were established by NMR spectral data using various 1D-NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ , and DEPT) and 2D-NMR (COSY, HMQC/HSQC, HMBC, H2BC, HSQC-TOCSY and NOESY) techniques, aided via UV, IR, MS data and also by comparison with data from the literature. Further isolation process of the plants yielded four types of isoquinoline alkaloid; aporphine, aporphine *N*-oxide, oxoaporphine and bisbenzylisoquinoline. A new aporphine *N*-oxide; norisocorydine *N*-oxide **166** with four aporphines; corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123**, were successfully isolated from samples that collected from Sg. Temau Reserve Forest. Samples from Sg. Tekam Reserve Forest, yielded four aporphines; corydine **124**, *N*-methylhernagine **169**, hernagine **123** and hernovine **170**, an oxoaporphine; oxohernagine **122** was isolated together with a bisbenzylisoquinoline; costaricine **126**. Antiplasmodial assay against *Plasmodium falciparum* on the bark crude extract exhibited active antiplasmodial activity with  $\text{IC}_{50}$  value 0.804  $\mu\text{g}/\text{ml}$ , while the isolated pure compounds corydine **124**, oxohernagine **122** and hernagine **123** showed the active antimalaria activity  $\text{IC}_{50}$  values at 1.184, 2.189 and 3.494  $\mu\text{M}$ , respectively. The new discovered compound, norisocorydine *N*-oxide showed a moderate effect on cytotoxic assay using leukaemia cell lines HL-60 with  $\text{IC}_{50}$  value 11  $\mu\text{g}/\text{ml}$ . Norisocorydine *N*-oxide **166**, corydine **124**, *N*-methylhernagine **169**, oxohernagine **122** and hernagine **123** exhibited very weak activity with  $\text{IC}_{50}$  values more than 500 $\mu\text{g}/\text{mL}$  against free radical scavenging effect of antioxidant DPPH assay and were not active against gram positive bacteria; *Bacillus subtilis* (B145). This is the first report for phytochemical and biological activity studies from barks of *Alseodaphne pendulifolia* Gamb., which has potential in determination of other various chemical constituents for new drug discovery.





## PEMENCILAN ALKALOID DARIPADA *Alseodaphne pendulifolia* Gamb. (LAURACEAE) DAN AKTIVITI-AKTIVITI BIOLOGI

### ABSTRAK

Kajian ini bertujuan untuk mengekstrak, memencil dan menentukan struktur sebatian alkaloid daripada kulit batang *Alseodaphne pendulifolia* Gamb. (Lauraceae) dan menentukan aktiviti biologi (antiplasmodial, sitotoksiti, antibakteria, dan antioksidan) terhadap sebatian alkaloid yang telah dipencarkan. Sampel diperoleh dari Hutan Simpan Sg. Temau, Kuala Lipis, Pahang dan Hutan Simpan Sg. Tekam, Jerantut, Pahang, Malaysia. Proses pemencilan dan penulenan alkaloid menggunakan pelbagai kaedah kromatografi termasuk kromatotron, kromatografi turus, kromatografi plat dan kromatografi lapisan nipis. Pengenalpastian struktur alkaloid yang telah dipencil dan ditentukan melalui data spektrum NMR menggunakan pelbagai teknik 1D-NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ , dan DEPT) dan 2D-NMR (COSY, HMQC/HSQC, HMBC, H2BC, HSQC-TOCSY dan NOESY), serta disokong oleh data daripada UV, IR, MS, dan juga melalui perbandingan data kajian literatur. Proses pemencilan sebatian kimia terhadap tumbuhan ini telah berjaya memperoleh empat alkaloid jenis isokuinolina; aporfina, aporfina *N*-oksida, oxoaporfina dan bisbenzilisokuinolina. Sebatian alkaloid aporfina *N*-oksida yang baru ditemui; norisokoridina *N*-oksida **166** dan empat lagi aporfina; koridina **124**, *N*-metilhernagina **169**, oxohernagina **122** dan hernagina **123** telah berjaya dipencarkan daripada sampel yang diperoleh dari Hutan Simpan Sg. Temau. Bagi sampel yang diperolehi dari Hutan Simpan Sg. Tekam, empat aporfina telah berjaya dipencarkan; koridina **124**, *N*-metilhernagina **169**, hernagina **123** dan hernovina **170**, satu oxoaporfina; oxohernagina **122** juga dipencarkan bersama-sama dengan satu sebatian bisbenzilisokuinolina; kostaricina **126**. Ujian antiplasmodial *Plasmodium falciparum* ke atas ekstrak mentah menunjukkan aktiviti antimalaria yang aktif dengan nilai IC<sub>50</sub> sebanyak 0.804 µg/ml, sementara untuk sebatian yang dipencarkan koridina **124**, oxohernagina **122** dan hernagina **123** menunjukkan aktiviti yang aktif iaitu nilai IC<sub>50</sub> masing-masing sebanyak 1.184, 2.189 and 3.494 µM. Ujian ketoksikdan terhadap sel leukimia HL-60, mendapati sebatian baru; norisokoridina *N*-oxide **166** menunjukkan kesan sederhana dengan nilai IC<sub>50</sub> 11 µg/ml. Sebatian norisokoridina *N*-oksida **166**, koridina **124**, *N*-metilhernagina **169**, oxohernagina **122** dan hernagina **123** menunjukkan aktiviti yang sangat lemah dengan nilai IC<sub>50</sub> melebihi 500 µg/mL terhadap kesan penjerapan radikal bebas dalam ujian antioksida DPPH dan juga menunjukkan kesan tidak aktif melawan bakteria gram positif: *Bacillus subtilis* (B 145). Ini merupakan laporan pertama mengenai kajian fitokimia dan aktiviti-aktiviti biologi terhadap kulit batang *Alseodaphne pendulifolia* Gamb. yang mana berpotensi untuk menemukan pelbagai jenis sebatian kimia yang lain untuk penghasilan ubat-ubatan baru.





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## LIST OF CHEMICAL COMPOUNDS STRUCTURE

No.	Chemical compounds structure name	Page
1	Citric acid	5
2	Tartaric acid	5
3	Malic acid	5
4	Cysteine	5
5	Tryptophan	5
6	Histidine	5
7	Serine	5
8	Alanine	5
9	Glutamic acid	6
10	Vanillin	6
11	Anthocyanins	6
12	Resveratrol	6
13	Morphine	6
14	Aspirin	6
15	Etoposide	6
16	Pyrethrins	6
17	Allethrins	6
18	Rotenone	7
19	Gibberellins	7
20	Cytokinins	7
21	Abscisic acid	7
22	Ethylene	7
23	Paclitaxel	20
24	Vitamin A	20
25	Vitamin D	21
26	Cantharidin	21
27	Doxorubicin	21
28	Tubocurarine	22
29	Cocaine	23
30	Codeine	23
31	Quinidine	23
32	Quinine	23
33	Vinblastine	23
34	L-ornithine	26
35	L-lysine	26
36	L-tyrosine	26
37	L-tryptophan	26
38	L-histidine	26
39	Usambarensine	26
40	Hordenine	26
41	Mescaline	26
42	Coniine	26
43	Capsaicin	27
44	Ephedrine	27
45	Solanidine	27
46	Caffeine	27





## LIST OF CHEMICAL COMPOUNDS STRUCTURE (conc.)

<b>47</b>	Theobromine	27
<b>48</b>	Pinidine	27
<b>49</b>	(S)-Reticuline	29
<b>50</b>	Norglaucine	29
<b>51</b>	Palmatine	30
<b>52</b>	Buesgeniine	30
<b>53</b>	Allocryptopine	30
<b>54</b>	Thebaine	30
<b>55</b>	Emetine	30
<b>56</b>	(+)-Stephasubine	30
<b>57</b>	Dopamine	34
<b>58</b>	4-hydrophenylacetadehyde	34
<b>59</b>	(S)-Norcochlurine	34
<b>60</b>	Corytuberine	35
<b>61</b>	Isoboldine	35
<b>62</b>	Glaucine	35
<b>63</b>	Bulbocapnine	35
<b>64</b>	Papaverine	34
<b>65</b>	Berberine	34
<b>66</b>	Sanguinarine	34
<b>67</b>	Erythraline	34
<b>68</b>	Argemonine	34
<b>69</b>	(S)-cochlaurine	38
<b>70</b>	<i>N</i> -methylcochlaurine	38
<b>71</b>	Berbamunine	38
<b>72</b>	Dauricine	40
<b>73</b>	Isoliensinine	40
<b>74</b>	Pisopowiardin	40
<b>75</b>	Imine cordobimine	40
<b>76</b>	Stebisimine	41
<b>77</b>	Sciaferine	41
<b>78</b>	Nortiliacorinine A	41
<b>79</b>	Cissampareine	41
<b>80</b>	1,2-Dehydromicranthine	42
<b>81</b>	Pseudorepanduline	42
<b>82</b>	Insulanoline	42
<b>83</b>	Dihydroisoobtusilactone	46
<b>84</b>	Dihydroobtusilactone	46
<b>85</b>	3-Epilitsenolide D <sub>1</sub>	46
<b>86</b>	3-Epilitsenolide D <sub>2</sub>	46
<b>87</b>	Alseodafuranone	46
<b>88</b>	Sanjoinine	46
<b>89</b>	(-)- <i>N</i> -norarmepavine	46
<b>90</b>	(+)-Reticuline	46
<b>91</b>	(+)-Coclaurine	47
<b>92</b>	(-)-Coclaurine	47
<b>93</b>	Lirioferine	47





## LIST OF CHEMICAL STRUCTURE (conc.)

<b>94</b>	Isocorydine	47
<b>95</b>	Norisocorydine	47
<b>96</b>	Obamegine	47
<b>97</b>	<i>N</i> -methyllaurotetanine	48
<b>98</b>	2-Norobamegine	48
<b>99</b>	Laurotetanine	48
<b>100</b>	(+)-2'-Noroxycanthine	48
<b>101</b>	(+)-Seeperine	48
<b>102</b>	(-)-Gyrolidine	49
<b>103</b>	(+)-Norstaphasubine	49
<b>104</b>	(+)-2-Norobaberine	49
<b>105</b>	<i>O</i> -Methyllymacusine	49
<b>106</b>	Norboldine	50
<b>107</b>	(+)-3',4'-Dihydrostaphasubine	50
<b>108</b>	<i>O</i> -Methylrepandine	50
<b>109</b>	Armepavine	50
<b>110</b>	Doryafranine	50
<b>111</b>	Xylopinine	51
<b>112</b>	1-(4-Methoxybenzyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline	51
<b>113</b>	4-Hydroxy, 3-methoxy benzoic acid	51
<b>114</b>	Neolignan eusiderin-A	51
<b>115</b>	Norlirioferine	51
<b>116</b>	Lindcarpine	51
<b>117</b>	Norpredicentrine	51
<b>118</b>	$\alpha'$ -oxoperakensimine	52
<b>119</b>	Boldine	52
<b>120</b>	<i>N</i> -(2-hydroxypropyl)-pendulifoline A	52
<b>121</b>	<i>N</i> -(2-hydroxypropyl)-pendulifoline B	52
<b>122</b>	Oxohernagine	52
<b>123</b>	Hernagine	52
<b>124</b>	Corydine	52
<b>125</b>	Isocorytuberine	53
<b>126</b>	Costaricine	53
<b>127</b>	Sebiferine	53
<b>128</b>	<i>N</i> -(2-hydroxypropyl)-norboldine A	53
<b>129</b>	<i>N</i> -(2-hydroxypropyl)-norboldine B	53
<b>130</b>	<i>N</i> -cyanomethyl-norboldine	53
<b>131</b>	$\alpha'$ -oxoperakensimine A	54
<b>132</b>	$\alpha'$ -oxoperakensimine B	54
<b>133</b>	$\alpha'$ -oxoperakensimine C	54
<b>134</b>	<i>N</i> -methyl-2, 3, 6-trimethoxy morphinandien-7-one <i>N</i> -oxide	54
<b>135</b>	<i>N</i> -methyl-2, 3, 6-trimethoxymorphinandien- 7-one	54
<b>136</b>	Perakensol (7-hydroxy-2, 3, 6- trimethoxyphenanthrene)	55
<b>137</b>	Srilankine	55
<b>138</b>	1,1-Diethoxy-ethane	55
<b>139</b>	1-Methyl-1H-pyrrole	55



**LIST OF CHEMICAL STRUCTURE (conc.)**

<b>140</b>	1,1-Diethoxy-2-methyl-propane	55
<b>141</b>	2-Methoxy-phenol	55
<b>142</b>	4-One,2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran	55
<b>143</b>	2-Methoxy-5-methylphenol	55
<b>144</b>	2-Methoxy-4-vinylphenol	55
<b>145</b>	D-allose	55
<b>146</b>	2,6-Dimethoxy-phenol	55
<b>147</b>	<i>N</i> -(2-morpholinoethyl)benzamide	55
<b>148</b>	1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane	55
<b>149</b>	Sucrose	56
<b>150</b>	1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-isoquinoline	56
<b>151</b>	2-Methoxy-4-(1-propenyl)-phenol	56
<b>152</b>	3,4,5-Trimethoxy-phenol	56
<b>153</b>	3,5-Dimethyl-2-nitro-phenol	56
<b>154</b>	2-Nonadecanone	56
<b>155</b>	2,2-Dimethyl-cyclohexanol	56
<b>156</b>	Tridecanoic acid methyl ester	56
<b>157</b>	3',5'-Dimethoxyacetophenone	56
<b>158</b>	( <i>E</i> )-3-Eicosene	56
<b>159</b>	2-Benzoyl-4-methoxymethyl-6-methyl-furo[2,3- <i>b</i> ]pyridin-3-amine	56
<b>160</b>	2-Oxo-hexadecanoic acid, methyl ester	56
<b>161</b>	5-Hydroxy-2,3,3-trimethyl-2-(3-methyl-but-1,3-dienyl)cyclohexanone	57
<b>162</b>	Di(3,4,methylenedioxy)benzylamine acetate	57
<b>163</b>	1-Hexyl-2-nitrocyclohexane	57
<b>164</b>	2-Methyl-3-(1-methylethenyl)-,acetate,(1 $\alpha$ ,2 $\alpha$ ,3 $\alpha$ )-cyclohexanol	57
<b>165</b>	(-)-1,2,3,4-Tetrahydro isoquinolin-6-ol-1-carboxylic acid, 7-methoxy-1-methyl-methyl ester	57
<b>166</b>	Norisocorydine <i>N</i> -oxide	82
<b>167</b>	Corydine <i>N</i> -oxide	85
<b>168</b>	Isocorydine <i>N</i> -oxide	85
<b>169</b>	<i>N</i> -methylhernagine	101
<b>170</b>	Hernovine	123





## ABBREVIATIONS

$^{13}\text{C}$ NMR	13-Carbon NMR
1D-NMR	One Dimension Nuclear Magnetic Resonance
$^1\text{H}$ NMR	Proton NMR
2D-NMR	Two Dimension Nuclear Magnetic Resonance
CC	Column Chromatography
$\text{CD}_3\text{OD}$	Deuterated methanol
$\text{CDCl}_3$	Deuterated chloroform
$\text{CH}_2\text{Cl}_2$	Dichloromethane
$\text{CH}_3$	Methyl group
$\text{CHCl}_3$	Chloroform
$\text{cm}^{-1}$	Per centimetre
COSY	$^1\text{H}$ - $^1\text{H}$ Correlation Spectroscopy
<i>D</i>	Doublet
<i>Dd</i>	Doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulphoxide
FT-NMR	Fourier Transform Nuclear Magnetic Resonance
G	Gram
HCl	Hydrogen chloride
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HREIMS	High Resolution Electron Spray Ionization Mass Spectroscopy





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## ABBREVIATIONS (conc.)

HRP2	Histidine-rich Protein 2
HSQC	Heteronuclear Single Quantum Correlation
Hz	Hertz
<i>J</i>	Coupling constant
kg	Kilogram
LC/MS	Liquid Chromatography – Mass Spectrometer
M	Molar
M	Meter
<i>m</i>	Multiplet
MeOH	Methanol
MHz	Mega Hertz
ml	Milliliter
mM	Milimolar
NH <sub>3</sub>	Ammonia
NMR	Nuclear Magnetic Resonance
OCH <sub>3</sub>	Methoxyl group
OH	Hydroxyl group
pH	Power of Hydrogen
Ppm	Parts per million
PTLC	Preparative Thin Layer Chromatography
<i>s</i>	Singlet



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## ABBREVIATIONS (conc.)

$t$	Triplet
TLC	Thin Layer Chromatography
$\alpha$	Alpha
$\beta$	Beta
$\delta$	Chemical shift
$\lambda$	Maximum wave length



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## CHAPTER 1

### INTRODUCTION



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Every single organism are made up by hundreds of thousands different chemical and each species producing its own characteristic mixture. Chemical composition in organism differ one to others, for example taste, scents, and colour of durian are totally different from apple because of its distinctive chemical composition. In spite of hundred or thousand of chemicals in the organism, each entities has their own ability of function either it was yet discovery or not by our modern scientists. But some different substances could share same properties (e.g., taste from citric acid **1** from citrus, tartaric acid **2** from grapes, and malic acid **3** from apples are perceived as sour by human tongue).



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In daily lives, besides of the varieties of plants in the world, delicious taste of fruits are beneficial to human body in various ways due to their distinctive chemical composition. In the Holy Quran, a number of fruits; banana (al-Waqi'ah [56]:28-33), dates (ar-Ra'd [13]:4), olive (an-Nahl [16]:10-11), pomegranate (al-'An'am [6]:141), fig (at-Tin [95]:1) and grapes (al-Mu'minun [23]:19) have been proven by modern science have their own nutritive value to prevent or cure various of disease (Al-Muammar & Khan, 2012; Biglari, AlKarkhi, & Easa, 2008; Bitler et al., 2007; Dueñas, Pérez-Alonso, Santos-Buelga, & Escribano-Bailón, 2008; Leifert & Abeywardena, 2008; Scarminio, Fruet, Witaicensis, Rall, & Di Stasi, 2012). In verses of al-'An'am ([6]:99), Allah S.W.T. commands human to observe and recount the variety and the beauty of His amazing creation with the obligation to protect sustainable environment (Azlan & Azad, 2012; *Holy Quran: English Translation by Abdullah Yusuf Ali*, 2000).



وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجَنَا بِهِ، نَبَاتٌ كُلُّ شَيْءٍ  
 فَأَخْرَجَنَا مِنْهُ خَضِرًا تُخْرِجُ مِنْهُ حَبَّاً مُتَرَاكِبًا وَمِنَ النَّخْلِ  
 مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّتٌ مِنْ أَعْنَابٍ وَالْزَيْتُونَ وَالرُّمَانَ  
 مُشْتَبِهًا وَغَيْرُ مُتَشَبِّهٍ قُمُّولُوا إِلَى ثَمَرَةٍ إِذَا أَثْمَرُ وَيَنْعِهٌ إِنَّ فِي  
 ذَلِكُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ

٩٩

*It is He Who sendeth down rain from the skies: with it We produce vegetation of all kinds: from some We produce green (crops), out of which We produce grain, heaped up (at harvest); out of the date-palm and its sheaths (or spathes) (come) clusters of dates hanging low and*





*near: and (then there are) gardens of grapes, and olives, and pomegranates, each similar (in kind) yet different (in variety): when they begin to bear fruit, feast your eyes with the fruit and the ripeness thereof. Behold! in these things there are signs for people who believe* (al-An'am [6]:99).

Each specific part of certain plant parts are produces numerous beneficial drugs products for mankind usage. Higher plants contribute about 25% of the total drugs in clinical use in the world. The specific plants organs that usually used likes herba or aerial parts (herba), leaf (folium), flower (flos), fruits (fructus), bark (cortex), root (radix), rhizome (rhizoma), and bulb (bulbus). The examples of variety plants parts that used as phytotherapeutic products are listed in Table 1.1.1(Skalicka-



Demand on natural products such as  $\alpha$ -amino acid demands over two million tons per year to produce various products that important in industries such as cysteine **4**, tryptophan **5**, and histidine **6** as antioxidants; serine **7** and alanine **8** are used in cosmetics; and the widely used as flavor enhancer; glutamic acid **9** or commonly known as monosodium glutamate (MSG) (Ahluwalia, Kumar, & Kumar, 2006). Nowadays, natural products were not only restricted to food industries, but they are widely applied in other field of usage such as beverages (vanillin **10**, anthocyanins **11**, and resveratrol **12**), pharmaceuticals (morphine **13**, aspirin **14**, and etoposide **15**), pesticides (pyrethrins **16**, allethrins **17**, and rotenone **18**) and plant growth regulators (gibberellins **19**, cytokinins **20**, abscisic acid **21** and ethylene **22**)(Chinou, 2008).





Table 1.1.1

*Specific Plants Parts in Certain Plants Species that Used in Phytotherapy.*

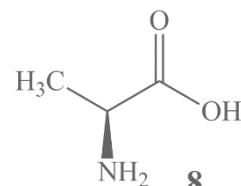
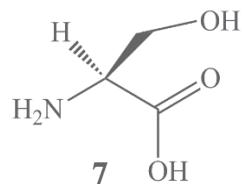
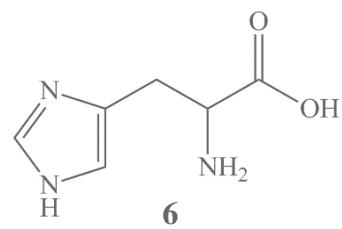
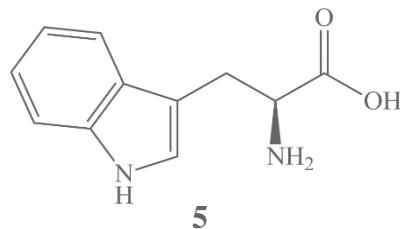
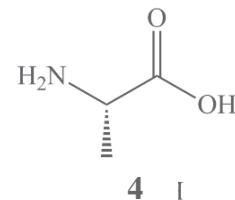
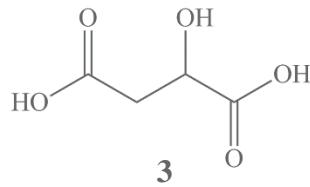
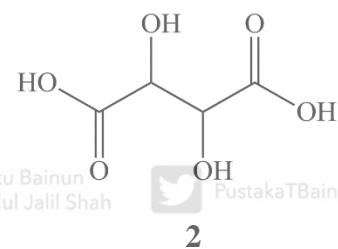
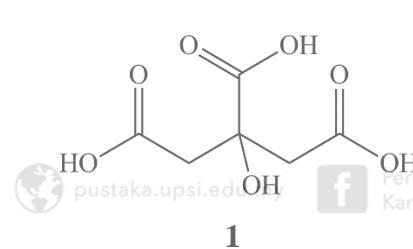
Plants parts	Plants
Fruits and seeds	Caraway ( <i>Carum carvi</i> ) Fennel ( <i>Foeniculum vulgare</i> ) Saw palmetto ( <i>Serenoa repens</i> ) Horse chestnut ( <i>Aesculus hippocastanum</i> )
Leaf	Balm ( <i>Melissa officinalis</i> ) Deadly nightshade ( <i>Atropa belladonna</i> ) Ginkgo ( <i>Ginkgo biloba</i> ) Peppermint ( <i>Mentha piperita</i> ) Bearberry ( <i>Arctostaphylos uva-ursi</i> )
Flowers	Chamomile ( <i>Chamomilla recutita</i> ) Calendula ( <i>Calendula officinalis</i> ) Arnica ( <i>Arnica Montana</i> )
Stem	Ephedra ( <i>Ephedra sinica</i> ) Hawthorn ( <i>Crataegus monogyna</i> ) Passion flower ( <i>Passiflora incarnata</i> ) Wormwood ( <i>Artemesia absinthium</i> )
Bark	Frangula ( <i>Rhamnus frangula</i> ) Willow ( <i>Salix alba</i> )
Rhizome and root	Devil's claw ( <i>Harpagophytum procubens</i> ) Tormentill ( <i>Potentilla erecta</i> ) Rhubarb ( <i>Rheum palmatum</i> ) Kava-kava ( <i>Piper methysticum</i> )

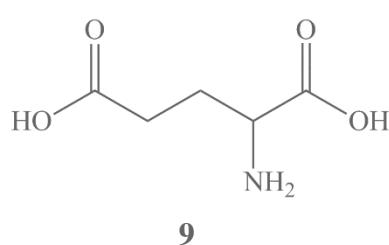
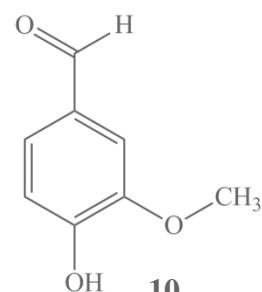
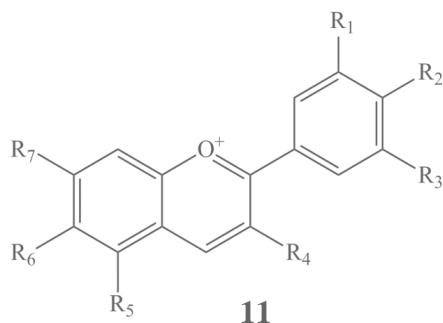
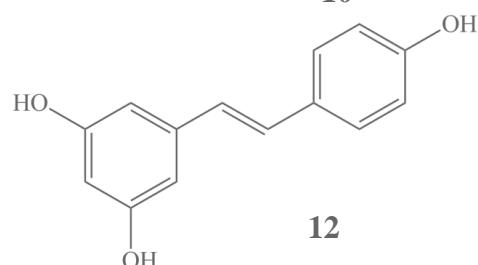
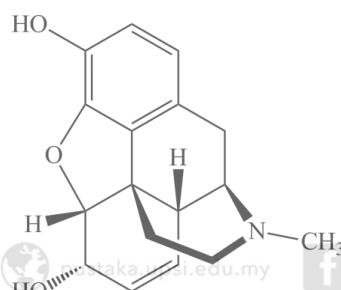
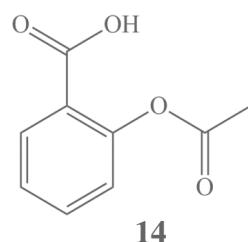
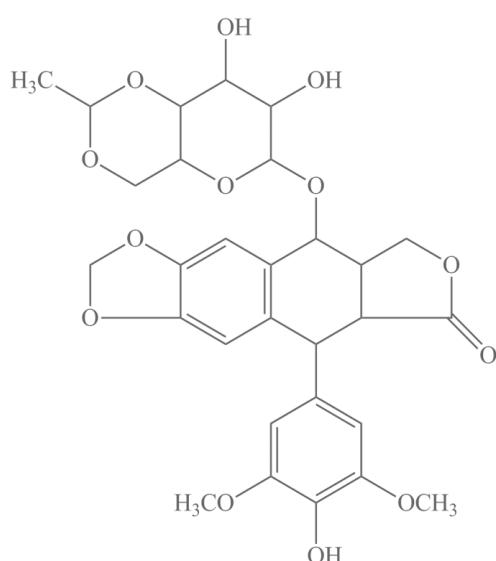
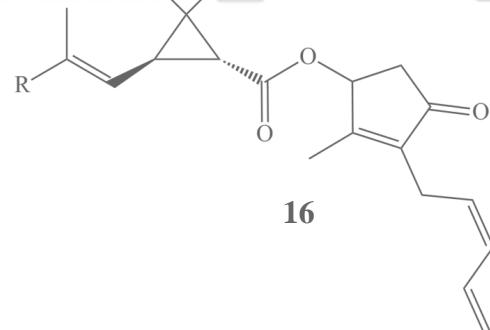
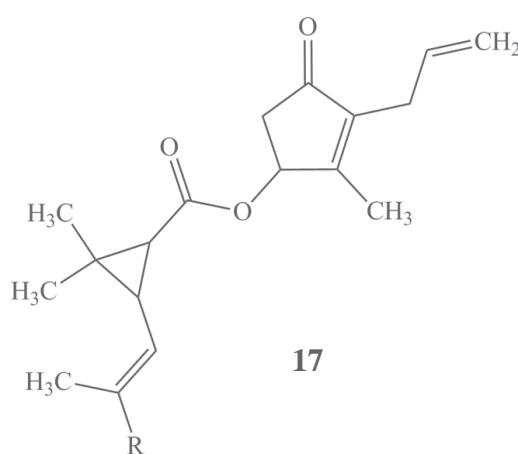
## 1.2 Problem Statements

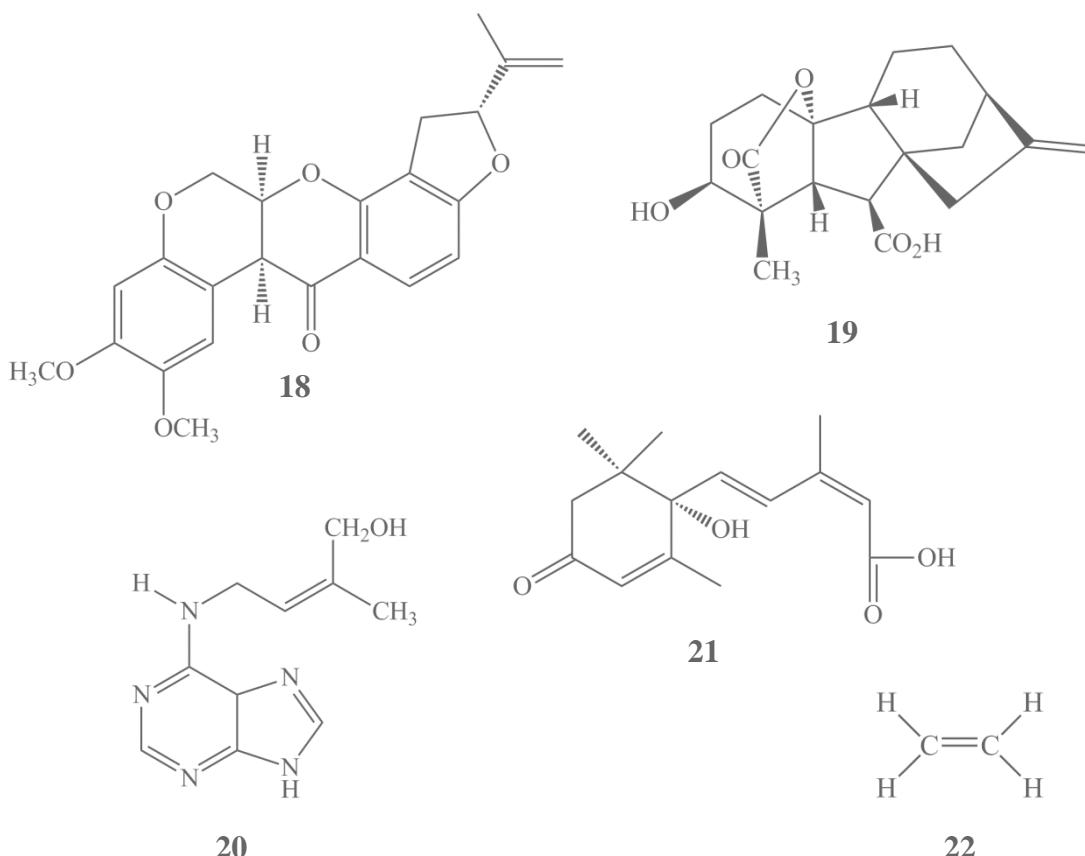
Malaysia is a tropical rain forest country which has been blessed with plethora of natural resources. Stand by its own, North Borneo indicated 3500 species of endemic plant which exhibiting high levels vascular plant species richness and endemism, while the other 2400 species thrives in Malaysian Peninsula (Ahmad et al., 2010; J.



Williams et al., 2001). Today, illegal logging activities for agriculture practice in our forest was endangered the flora and the might be extinct within time before our phytochemist and pharmacist could discover their chemical constituents and potential as our new drugs discovery. A fast action must be taken to overcome this problem. This study will provide a new literature about alkaloid constituents and their biological activities from *Alseodaphne pendulifolia* Gamb. that collected from two different locations. This is the first report that study of alkaloid constituents from bark of this species. From the literature review, not much papers are reported about chemical constituents from genus of *Alseodaphne* (Thakur et al., 2012).



**9****10****11****12****13****14****15****16****17**



### 1.3 Objectives of Study

The objectives of the study are as follows:

- To extract, isolate and purify alkaloid constituents from barks of *Alseodaphne pendulifolia* Gamb.
- To identify and elucidate the structure of the isolated alkaloids constituent using modern spectroscopy methods.
- To determine the antiplasmodial, cytotoxicity, antibacterial and antioxidant activities against the isolated alkaloids from the barks of *Alseodaphne pendulifolia* Gamb.



## 1.4 Family of Lauraceae

The Lauraceae are predominantly arboreous family, a large pantropical family mainly composed of large trees or shrubs that mostly found in warm, tropical, humid subtropical and mild temperate regions of northern and southern hemispheres (Joshi, Verma, & Mathela, 2010; Simie et al., 2004). Sixty eight genera and more than two thousand species have been reported under this family (Drewes, Horn, & Mavi, 1997; Kumar, Rawat, Rahuja, Srivastava, & Maurya, 2009; Little, Stockey, & Penner, 2009; Oh et al., 2012; “The plant list, a working list of all known plant species.,” 2010). The taxonomic hierarchy of Lauraceae family have been enlisted below:

Domain : Eukaryota



Division : Magnoliophyta

Class : Magnoliopsida

Order : Laurales

Family : Lauraceae

Genera :

1. Actinodaphne
2. Aiouea
3. Alseodaphne
4. Aniba
5. Apollonias
6. Aspidostemon

7. Beilschmiedia
8. Camphora
9. Caryodaphnopsis
10. Cassytha
11. Chlorocardium
12. Cinnadenia





- |                   |                         |
|-------------------|-------------------------|
| 13. Cinnamomum    | 38. Neolitsea           |
| 14. Cryptocarya   | 39. Nesodaphne          |
| 15. Dehaasia      | 40. Notaphoebe          |
| 16. Dicypellium   | 41. Nothaphoebe         |
| 17. Dodecadenia   | 42. Ocotea              |
| 18. Endiandra     | 43. Oreodaphne          |
| 19. Endlicheria   | 44. Paraia              |
| 20. Eusideroxylon | 45. Parasassafras       |
| 21. Gamanthera    | 46. Parthenoxylon       |
| 22. Hufelandia    | 47. Persea              |
| 23. Hypodaphnis   | 48. Phoebe              |
| 24. Iteadaphne    | 49. Phyllostemonodaphne |
| 25. Kubitzkia     | 50. Pleurothyrium       |
| 26. Laurus        | 51. Polyadenia          |
| 27. Licaria       | 52. Potameia            |
| 28. Lindera       | 53. Potoxylon           |
| 29. Litsea        | 54. Povedadaphne        |
| 30. Machilus      | 55. Ravensara           |
| 31. Malapoenna    | 56. Rhodostemonodaphne  |
| 32. Mespilodaphne | 57. Sassafras           |
| 33. Mezilaurus    | 58. Schauera            |
| 34. Mocinnodaphne | 59. Sextonia            |
| 35. Mutisiopersea | 60. Sinopora            |
| 36. Nectandra     | 61. Sinosassafras       |
| 37. Neocinnamomum | 62. Syndiclis           |





63. Systemonodaphne

66. Urbanodendron

64. Tetrantha

67. Williamodendron

65. Umbellularia

68. Yasunia

This family is largely characterized by trimerous flowers, bi- or tetrasporangiate anthers with apical valvate dehiscence, and a unicarpellate gynoecium containing a single anatropous, apically attached ovule. Fruits of the family are fleshy, typically black to almost black at maturity and usually borne on enlarged receptacles that can form cupulate to fully enclosing structures. Fruits of Lauraceae are typically considered drupes, but are better classified as a berry since the “endocarp” is only made up of a thin, single-celled sclerified endodermis. Oil or mucilage idioblasts occur throughout the plant tissues, including the wood (Little et al., 2009).



Name of Lauraceae itself is derived from the prominent member, the Grecian laurel, *Laurus nobilis*, is characterized by plants which have prominent oil cells in the leaves, wood and fruits. These oils are mostly aromatic hence provide a number of flavouring materials and spices (Schroeder, 1975). Recorded showed that the lauraceous plants have widespread use in both oriental medicine and occidental medicine (Shimonura, Sashida, Mimaki, Oohara, & Fukai, 1988). Some plants in this family have been employed in folk medicine for their interesting bioactivities (Rachmatiah, Mukhtar, Nafiah, Hanafi, Awang, et al., 2009; Rachmatiah, Mukhtar, Nafiah, Hanafi, Kosela, et al., 2009). *Cinnamomum camphora* (L.) Presl is a major source of camphor, which can be made into camphor oil and mothballs. In addition, camphor is taken orally to calm hysteria, nervousness, neuralgia and to treat serious diarrhoea. Camphor is also known to be effective in treating colds and chills ( Lee et





al., 2006). The extracts from *C. Cassia* have been claimed to reduce inflammation (Lee & Shibamoto, 2002) and to decrease serum glucose, total cholesterol and platelet counts (Khan, Safdar, Ali-Khan, Khattak, & Anderson, 2003). Table 1.3.1 showed uses of species from family of Lauraceae.

Table 1.3.1

*Uses of Species from Family of Lauraceae*

Uses	Species of Lauraceae	References
Spices	<i>Cinnamomum cassia</i> <i>Cinnamomum subavenium</i> <i>Cinnamomum zeylanicum</i> <i>Laurus nobilis</i> <i>Sassafras albidum</i>	(Gottlieb, 1972; Kumar et al., 2009; Perera & Rajapakse, 1991)
Nutritious fruits	<i>Persea americana</i>	(Gottlieb, 1972; Kumar et al., 2009; Perera & Rajapakse, 1991)
Chinese folk medicines	<i>Cinnamomum cassia</i> <i>Lindera aggregate</i>	(Kumar et al., 2009)
Timber	<i>Eusideroxylon zwageri</i> <i>Mezilaurus itauba</i> , <i>Mezilaurus navalium</i> <i>Ocotea bullata</i> <i>Persea nanmu</i>	(Gottlieb, 1972)
Seed fat	<i>Laurus nobilis</i> <i>Litsea sebifera</i>	(Gottlieb, 1972)
Perfume oils	<i>Aniba duckei</i> <i>Aniba rosaeodora</i> <i>Ocotea pretiosa</i>	(Gottlieb, 1972)





## 1.5 Genus of *Alseodaphne*

Genus of *Alseodaphne* consist more than fifty species (Table 1.4.1), distributed around Malaysia, China, Cambodia, Vietnam, Laos, Indonesia, Sri Lanka, Myanmar, Philippines, Thailand and India. This genus locally known as medang or tejur in Malaysia (International Seed Testing Association, 2013; Omar, Nafiah, Mukhtar, Awang, & Hadi, 2009), Wewarana in Sri Langka (Ariyadasa, 2002), Gemur in Indonesia (Joosten, Tapio-Bistrom, & Tol, 2012; Kalimantan Forests and Climate Partnership, 2009) and Nelthare in Tamil Arambamaram, Kanaippirandai (Alex, Charles, Leo, Joseph, & Mani, 2012; Charles, Stanly, Joseph, & Ramani, 2011).

From more than sixty species, only a few from this species were reported their

usage. The trees of *Alseodaphne semecarfolia* was reported used as nest by Hornbill (Mudappa & Kannan, 1997; Velho, Datta, & Isvaran, 2009) and fruits was eaten as one of their food sources in Sg. Tekam Forest Reserve (Andrew D. Johns, 1987) but for *Alseodaphne perakensis*, aborigines of the Sakai tribe claimed that the fruit from this plant is poisonous (Lajis, Mahmud, Din, & Toia, 1989). The versatile and aesthetic qualities hardwood of *Alseodaphne hainanensis*, *A.oblanceolata* and *A. perakensis*, are highly demand in specific market of “luxury” applications including buildings, furniture, ships and ornaments due to their quality of hue, grain, texture, strength and durability (Huang, 2011; Lajis et al., 1989; Schroeder, 1982). In construction sector, the durability of *A. dura* and *A. longipes* is leveraged as seawater piles (Schroeder, 1982).





In Sumatra, Indonesia, *A. coriacea* is a livelihood sources for some Sumatran. It was harvested in deep forest for their valuable bark that rich with pyrethrins **16**, a compound that used in variety of insecticides especially in production of mosquito coil. Besides insecticides, the bark of this species also used in production of joss sticks (dupa cina), surfactant for recycling engine oil and some in cosmetics products (Janudianto, Mulyoutami, Joshi, Wardell, & Noordwijk, 2011; Joosten et al., 2012; Kristedi & Kieft, 2010; Lyons, 2003). In folk medicine, *A. semicarpifolia* was used as treatment of some illness likes cholera-like illness, rinderpest and dysentery diseases in cattle and also bark juice is applied externally for leach bite or chest pain (Alex et al., 2012; *Basic Medical Sciences; Intramural Research: An Annual Report*, 2012; Charles, Joseph, & Ramani, 2012; Charles et al., 2011; Harsha, Shripathi, & Hegde, 2005; Karuppusamy, 2007; Parinitha, Harish, Vivek, Mahesh, & Shivanna, 2004)



Alseodaphne are classified as evergreen trees. The leaves were alternate, always clustered near apex of branchlet, pinninerved, often turning black when dry. Inflorescence axillary, paniculate or racemose; bracts and bracteoles deciduous. Flowers are bisexual and trimerous. Perianth tube short; perianth lobes, subequal or outer is smaller, slightly dilated after anthesis but absent in fruit. Fertile stamens, in three whorls; filaments of first and second whorls glandless, those of third whorl each with two glands at base; anthers four-celled; cells of first and second whorls introrse, those of third whorl extrorse or upper two lateral and lower two extrorse. Staminodes of innermost whorl, very small, nearly sagittate. Ovary partly immersed into shallow perianth tube; style often as long as ovary; stigma small, inconspicuous, discoid. Fruit black or purplish black when mature, ovoid, oblong, or subglobose; fruit stalk red,





green, or yellow, sometimes nearly cylindric, fleshy, pulpy, always warty, truncate at apex (Shu, Hsi-wen, Jie, & Werff, 2008).

Table 1.4.1

*List of Species in Genus of Alseodaphne (Lauraceae)*

No.	Species	References
1	<i>A. albifrons</i>	I,II,III
2	<i>A. andersonii</i>	II,IV,V
3	<i>A. archboldiana</i>	VI
4	<i>A. bancana</i>	II,VII,VIII
5	<i>A. birmanica</i>	II
6	<i>A. borneensis</i>	II
7	<i>A. cavaleriei</i>	II
8	<i>A. ceratoxylon</i>	IX,X
9	<i>A. changchangensis</i>	IV
10	<i>A. coriacea</i>	I,XI,XII
11	<i>A. corneri</i>	II,XIII
12	<i>A. costalis</i>	VIII
13	<i>A. cuneata</i>	X
14	<i>A. dura</i>	II,XIV
15	<i>A. elmeri</i>	II
16	<i>A. elongata</i>	II
17	<i>A. falcate</i>	X
18	<i>A. foxiana</i>	II





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19	<i>A. garciniicarpa</i>	<b>II</b>
20	<i>A. gigaphylla</i>	<b>IV</b>
21	<i>A. glauciflora</i>	<b>II</b>
22	<i>A. glaucina</i>	<b>II</b>
23	<i>A. gracilis</i>	<b>II,V</b>
24	<i>A. grandis</i>	<b>VIII</b>
25	<i>A. griffithii</i>	<b>II</b>
26	<i>A. habrotricha</i>	<b>II</b>
27	<i>A. hainanensis</i>	<b>II,IV,V,XV,XVI,XVII</b>
28	<i>A. havilandii</i>	<b>X</b>
29	<i>A. himalayana</i>	<b>II</b>
30	<i>A. hokouensis</i>	<b>II,IV,V</b>
31	<i>A. huanglianshanensis</i>	<b>II,IV,V</b>
32	<i>A. insignis</i>	<b>I,II,IX,X,XVIII</b>
33	<i>A. intermedia</i>	<b>I,II,VII,XIX</b>
34	<i>A. khasyana</i>	<b>II</b>
35	<i>A. kochummenii</i>	<b>II</b>
36	<i>A. langugenosa</i>	<b>XIV</b>
37	<i>A. lanuginosa</i>	<b>II</b>
38	<i>A. longipes</i>	<b>II,XIV,XVIII</b>
39	<i>A. marlipoensis</i>	<b>II,IV,V</b>
40	<i>A. medogensis</i>	<b>II</b>
41	<i>A. micrantha</i>	<b>II</b>
42	<i>A. montana</i>	<b>II</b>

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43	<i>A. nigrescens</i>	<b>II,IV,VII,XX</b>
44	<i>A. oblanceolata</i>	<b>II,XIV,XIX</b>
45	<i>A. obovata</i>	<b>I</b>
46	<i>A. owdenii</i>	<b>II</b>
47	<i>A. paludosa</i>	<b>II,X</b>
48	<i>A. peduncularis</i>	<b>I,II</b>
49	<i>A. pendulifolia</i>	<b>I,II</b>
50	<i>A. perakensis</i>	<b>II,XIII,XIX,XXI,XXII,XXIII,XXIV</b>
51	<i>A. petiolaris</i>	<b>II,IV,V,XXV,XXVI,XXVII</b>
52	<i>A. philippinensis</i>	<b>II</b>
53	<i>A. ramosii</i>	<b>II</b>
54	<i>A. rhododendropsis</i>	<b>II</b>
55	<i>A. ridleyei</i>	<b>I,II</b>
56	<i>A. rubriflora</i>	<b>II</b>
57	<i>A. rubrolignea</i>	<b>II</b>
58	<i>A. rugosa</i>	<b>II,IV,V,XXVIII</b>
59	<i>A. semecarpifolia</i>	<b>II,IV,XXI,XXIX,XXX,XXXI,XXXII,XXXIII,X XXIV,XXXV,XXXVI,XXXVII,XXXVIII,XXXI X,XL,XLI</b>
60	<i>A. siamensis</i>	<b>II</b>
61	<i>A. sichourensis</i>	<b>II,IV,V</b>
62	<i>A. suboppositifolia</i>	<b>II</b>
78	<i>A. sulcata</i>	<b>II</b>
64	<i>A. tomentosa</i>	<b>II</b>
65	<i>A. tonkinensis</i>	<b>II</b>

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66	<i>A. utilis</i>	II
67	<i>A. umbaliflora</i>	XVIII,XLII
68	<i>A. wrayi</i>	II
69	<i>A. yunnanensis</i>	II,V

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References : **I** (International Seed Testing Association, 2013), **II** (Thakur et al., 2012), **III** (Zulkifly, Kim, Majid, & Merican, 2011), **IV** (Li et al., 2011), **V** (Shu et al., 2008), **VI** (Bick, 1996), **VII** (Chong, Tan, & Corlett, 2009), **VIII** (Wong et al., 2013), **IX** (Kartawinata et al., 2006b), **X** (Kartawinata et al., 2006a), **XI** (Joosten et al., 2012), **XII** (Kalimantan Forests and Climate Partnership, 2009), **XIII** (Sulaiman et al., 2011), **XIV** (Schroeder, 1982), **XV** (Huang, 2011), **XVI** (Ortega et al., 2010), **XVII** (Zhang & Ma, 2008), **XVIII** (Hidayat & Simpson, 1994), **XIX** (Ya'akub, Kudus, Nazre, Noor, & Hanum, 2013), **XX** (Tata, Noordwijk, & Werger, 2008), **XXI** (Lajis et al., 1989), **XXII** (Mahmud, 1989), **XXIII** (Nafiah et al., 2011), **XXIV** (Okechukwu, 2005), **XXV** (Lan, Zhu, & Cao, 2012), **XXVI** (Majumdar, Datta, & Shankar, 2012), **XXVII** (Rout, Sajem, & Nath, 2012), **XXVIII** (Koskela, Hong, & Rao, 2002), **XXIX** (Alex et al., 2012), **XXX** (Ariyadasa, 2002), **XXXI** (Chanderbali, Werff, & Renner, 2001), **XXXII** (Charles & Alex, 2011), **XXXIII** (Charles et al., 2012), **XXXIV** (Charles et al., 2011), **XXXV** (Harsha et al., 2005), **XXXVI** (Jayakumar, Arockiasamy, & Britto, 2000), **XXXVII** (Karuppusamy, 2007), **XXXVIII** (Mahadkar, Valvi, & Jadhav, 2013), **XXXIX** (Mudappa & Kannan, 1997), **XL** (Parinitha et al., 2004), **XLI** (Parthasarathy, 1999), and **XLII** (Adinugroho, Suryadiputra, Saharjo, & Siboro, 2011)



## 1.6 Species of *Alseodaphne pendulifolia* Gamb.

*Alseodaphne pendulifolia* Gamb. (Figure 1.5.1 – 1.5.3) is a medium to large sized tree with approximately 120 cm trunk circumference, height up to 18 m tall, that are rarely found in lowland forests around Perak, Terengganu, Pahang, Selangor, and Borneo Island. This plant has pale yellow flowers in auxiliary panicles, with perianth glabrous outside and outer whorl smaller than inner. The shape of fruits are ovoid with 1.5 x 1 cm in sized with about 15 cm long perianth tube which slightly enlarged (Nafiah, 2009; Ng, 1989; Thakur et al., 2012).





The colour of the bark is brownish, smooth to lenticels parts and the inner bark are yellow same as sapwood. The twigs are thick with prominent leaf scars. The leaves are clustered at the ends of the twigs, dropping down with stalk about 2 - 4 cm along and blade leathery, slightly glaucous below, obovate or oblanceolate shape. The apex is blunt or pointed and the base is narrowed. Midrib rose above and the secondary nerves are about 15-22 pairs, curving and joining near margin, which is visible on above side (Nafiah, 2009; Ng, 1989; Thakur et al., 2012).



Figure 1.5.2: Barks of *Alseodaphne pendulifolia* Gamb.



Figure 1.5.3: Leaves of *Alseodaphne pendulifolia* Gamb.

