

TABLE OF CONTENTS

	Page
Acknowledgments	iv
Abstract	vi
List of Tables	xv
List of Figures	xviii
List of Schemes	xx
List of Spectra	xxi
List of Appendices	xxii
List of Abbreviations	xxiii
 CHAPTER 1 – INTRODUCTION	 1
 1.1. General Considerations	 2
1.2. Chemotaxonomy	4
1.3. Family Leguminosae Juss.	5
1.4. Taxonomy of the Leguminosae	7
1.5. Chemistry of the Leguminosae	8
1.6. Subfamily Papilionoideae	8
1.7. Tribe Millettieae	9
1.8. The Genus <i>Lonchocarpus</i> HBK	12
1.8.1. Ethnobotanical and Ethnomedical Information on <i>Lonchocarpus</i> Species in Mexico	16
1.8.2. Previous Phytochemical Investigations on <i>Lonchocarpus</i> Species	16
1.8.3. <i>Lonchocarpus xuul</i> Lundell	55
1.8.4. <i>Lonchocarpus yucatanensis</i> Pittier	57
1.9 The Genus <i>Pithecellobium</i> C. Martius	59
1.9.1. Previous Phytochemical Investigations on <i>Pithecellobium</i> Species	60
1.9.2. Previous Pharmacological Investigations on <i>Pithecellobium</i> Species	71
1.9.3. Ethnobotanical and Ethnomedical Information on Plants in the genus <i>Pithecellobium</i>	71
1.9.4. <i>Pithecellobium albicans</i> (Kunth) Benth	72

	Page
CHAPTER 2 – MATERIALS AND METHODS	74
2.1. Plant Materials	75
2.2. Extractions Techniques	75
2.3. General Methodology	79
2.3.1. Chromatographic Methods	79
2.3.1.1. Analytical Thin Layer Chromatography	79
2.3.1.2. Preparative Thin Layer Chromatography	79
2.3.1.3. Vacuum Liquid Chromatography	80
2.3.1.4. Open Column Chromatography	80
2.3.1.5. Flash Chromatography	81
2.3.1.6. Gel Permeation Chromatography	81
2.3.2. Detection	81
2.3.2.1. UV Detection	81
2.3.2.2. Chemical Detection	82
2.3.2.2.1. Vanillin in H ₂ SO ₄	82
2.3.2.2.2. Phosphomolybdic Acid	82
2.3.2.2.3. Ammonia Vapour	82
2.3.3. Derivatization	82
2.3.3.1. Acetylation	82
2.3.3.2. Acetonide Formation	83
2.3.4. Compounds Isolated from the Species Studied	83
2.4. Structure Elucidation	92
2.4.1. Physical Data	92
2.4.2. Spectral Data	92
2.4.2.1. UV-Visible (UV-Vis)	92
2.4.2.2. Mass Spectra (MS)	92
2.4.2.3. Infrared Spectra (IR)	93
2.4.2.4. Nuclear Magnetic Resonance (NMR)	93
2.4.3. NMR Techniques	94
2.4.3.1. One Dimensional NMR	94
2.4.3.1.1. ¹ H NMR	94
2.4.3.1.2. <i>J</i> -modulated ¹³ C NMR	94

	Page
2.4.3.2. Two Dimensional NMR Techniques	95
2.4.3.2.1. Two Dimensional Homonuclear Experiments	97
2.4.3.2.1.1. COSY	97
2.4.3.2.1.2. NOESY	97
2.4.3.2.2. Two Dimensional Heteronuclear Experiments	97
2.4.3.2.2.1. HMQC	97
2.4.3.2.2.2. HMBC	97
2.5. Cytotoxicity Assay by ATPLite-M	98
2.5.1. Principle	98
2.5.2. Materials	98
2.5.3. Sample Preparation	99
2.5.4. Assay Procedure	99
2.5.5. Inhibitory Concentration at 50% (IC ₅₀) Determination	99
2.6. Cell Cycle Method	100
2.6.1. Materials	100
2.6.2. Sample Preparation	100
2.6.3. Cells Preparation	100
2.6.4. Assay Procedure	100
 CHAPTER 3 – PHYTOCHEMICAL RESULTS AND DISCUSSION	 102
 3.1. Flavonoids	 103
3.1.1. Chalcones	103
3.1.1.1. Characterization of the novel 2', 4-dimethoxy-lonchocarpin (180)	104
3.1.1.2. Identification of chalcone (196) as 2',4'-dihydroxy-3'-(3-methylbut-2-enyl) chalcone (Isocordoin, Flemistricin A)	109
3.1.2. Flavanones	113
3.1.2.1. Identification of flavanone (191) as 5,7-dihydroxy-6, 8-di- (3-methylbut-2-enyl) flavanone (Spinoflavanone B)	114
3.1.2.2. Characterization of 5-hydroxy- (6:7)-2,2-dimethylpyranoflavanone (199)	117

	Page
3.1.3. Flavones	120
3.1.3.1. Characterization of 5,4'-dimethoxy-(6:7)-2,2-dimethylpyranoflavone (182)	122
3.1.3.2. Characterization of 5,4'-dihydroxy, 3'-methoxy-(6:7)-2,2-dimethylpyranoflavone (183)	126
3.1.3.3. Identification of flavone (184) as 5,4'-dihydroxy-(6:7)-2,2-dimethylpyranoflavone	129
3.1.4. Flavonol Glycosides	130
3.1.4.1. Identification of Flavonol (185) as Quercetin-3- <i>O</i> -rutinose	132
3.1.4.2. Identification of Flavonol (186) as Kaempferol-3- <i>O</i> -rhamnoglucoside	139
3.1.4.3. Identification of Flavonol Glycoside (202) as Kaempferol-3- <i>O</i> -rhamnoside	140
3.1.4.4. Identification of Flavonol Glycoside (203) as Quercetin-3- <i>O</i> -rhamnoside	141
3.1.5. Flavans	142
3.1.5.1. General characterization of isolated flavans	143
3.1.5.2. Characterization of 4 β ,5-dimethoxy-(6:7)-2,2-dimethylpyranoflavan (190)	146
3.1.5.3. Characterisation of 3 β , 4 β , 5-trimethoxy- (6:7)-2,2-dimethylpyranoflavan (192)	152
3.1.5.4. Characterisation of 4 β -ethoxy-5-methoxy-(6:7)-2,2-dimethylpyranoflavan (193)	152
3.1.5.5. Characterisation of 4 β ,5-dimethoxy-3 β -hydroxy- (6:7)-2,2-dimethylpyranoflavan (194)	154
3.1.5.6. Characterisation of 3 β , 4 β -dihydroxy-5-methoxy-(6:7)-2,2- dimethylpyranoflavan (195)	156
3.1.5.7. Characterisation of 3,4-epoxy-5-methoxy- (6:7)-2,2-dimethylpyranoflavan (200)	156
3.1.5.8. Characterisation of 3 β , 4 β -5-trimethoxy-4'-hydroxy-(6:7)-2,2-dimethylpyranoflavan (197)	161
3.1.6. Flavens and the Characterization of 5-Methoxy- (6:7)-2,2-dimethylpyrano-flav-3-ene (189)	165

	Page
3.1.7. Properties and Spectral Data of Isolated Flavonoids	168
3.1.7.1. 2'4-dimethoxylonchocarpin (189)	168
3.1.7.2. 2',4'-dihydroxy-3'-(3-methylbut-2-enyl)chalcone (196)	168
3.1.7.3. 5,7-dihydroxy-6, 8-di (3-methylbut-2-enyl) flavanone (Spinoflavanone B) (191)	168
3.1.7.4. 5-hydroxy- (6:7)-2,2-dimethylpyranoflavanone (199)	168
3.1.7.5. 5,4'-dimethoxy-(6:7)-2,2-dimethylpyranoflavone (182)	169
3.1.7.6. 5,4'-dihydroxy-3'-methoxy- (6:7)-2,2-dimethylpyranoflavone (183)	169
3.1.7.7. 5,4'-dihydroxy-(6:7)-2,2-dimethylpyranoflavone (184)	169
3.1.7.8. Quercetin-3-O-rutinoside (185)	169
3.1.7.9. Quercetin-3-O-rutinoside acetate (187)	169
3.1.7.10. Kaempferol-3-O-rutinoside (186)	170
3.1.7.11. Kaempferol-3-O-rhamnoside (202)	170
3.1.7.12. Quercetin-3-O-rhamnoside (203)	170
3.1.7.13. 4β-5-Dimethoxy-(6,7)-2,2-dimethylpyranoflavan (190)	170
3.1.7.14. 3β,4β,5-Trimethoxy- (6:7)-2,2-dimethylpyranoflavan (192)	171
3.1.7.15. 4β-Ethoxy-5-methoxy-(6:7)-2,2-dimethylpyranoflavan (193)	171
3.1.7.16. 4β-5-Dimethoxy-3β-hydroxy- (6:7)-2,2-dimethylpyranoflavan (195)	171
3.1.7.17. 3β-Acetoxy-4β, 5-dimethoxy- (6:7)-2,2-dimethylpyranoflavan (198)	171
3.1.7.18. 3β, 4β-dihydroxy-5-methoxy-(6:7)-2,2-dimethylpyranoflavan (195)	172
3.1.7.19. 3β, 4β-dihydroxy-5-methoxy-(6:7)-2,2-dimethylpyranoflavan-3, 4-acetonide (201)	172
3.1.7.20. 3β, 4β-5-trimethoxy-4'-hydroxy-(6:7)-2,2-dimethylpyranoflavan] (197)	172
3.1.7.21. 3β, 4β-epoxy-5-methoxy-(6:7)-2,2-dimethylpyranoflavan (200)	173
3.1.7.22. 5- Methoxy- (6:7)-2,2-dimethylpyranoflav- 3-ene (189)	173

	Page
3.2. Amino Acids	174
3.2.1. Identification of Metabolite (188) as 4-Hydroxy-N-methylproline	174
3.2.2. Properties and Spectral Data of Isolated Amino Acid (188)	175
3.3. Terpenoids	180
3.3.1. Identification of metabolite AB-4B as β -sitosterol	180
3.3.2. Identification of Fraction AB-4D as Mixture of Lupeol and β -Amyrin	181
3.3.3. Identification of metabolite AB-5A as β -amyrenone	182
3.3.4. Properties and Spectral Data of Isolated Triterpenoids	184
3.3.4.1. β -Sitosterol (AB-4B)	184
3.3.4.2. β -amyrenone (AB-5A)	184
3.4. Miscellaneous Compounds	186
3.4.1. Identification of <i>p</i> -Coumaric Acid Methyl Ester (181)	186
3.4.2. Properties and Spectral Data of <i>p</i> -Coumaric Acid Methyl Ester (181)	186
 CHAPTER 4 - BIOLOGICAL ACTIVITY ASSAY AND DISCUSSION	 188
 4.1. ATP Luminescence Assay	 189
4.2. Cell Cycle Assay	190
4.3. Conclusions on Biological Activity of Metabolites Isolated	192
 CHAPTER 5 - CHEMOTAXONOMIC DISCUSSION	 193
 5.1. Flavonoids as Systematic Markers in the Genus <i>Lonchocarpus</i>	 194
5.1.1. Prenylation patterns	194
5.1.2. Oxygenation Patterns	206
5.1.3. Isoflavonoids	208
5.2. Discussion of Chemical Systematics in <i>Lonchocarpus</i>	210
5.2.1. Comparative Phytochemistry of <i>Lonchocarpus xuul</i> Lundell and <i>Lonchocarpus yucatanensis</i> Pittier	210

	Page
5.2.2. Flavonoid Patterns and Classification in the Genus <i>Lonchocarpus</i>	214
5.2.2.1. Flavonoid Types and Substitution Patterns Observed in the Genus	215
5.2.2.1.1. Analysis Based on Traditional Classification (Table 5.13)	215
5.2.2.1.2. Analysis Based on the Geographical Location (Distribution) of Species (Table 5.14)	215
5.2.2.1.3. Analysis Based on the Plant Part Studied	215
5.2.2.1.4. Analysis Based on Flavonoid Substitution Pattern	216
5.2.2.1.5. Analysis Based on Flavonoid Type (s) Pattern (Table 5.17)	216
5.3 Conclusions on Chemotaxonomy of the Genus Lonchocarpus	216
REFERENCES	228