

**CHEMICAL CHARACTERISTICS,  
ANTIOXIDANT AND ANTIFUNGAL  
ACTIVITIES OF *CASSIA FISTULA* LINN. AT  
DIFFERENT TREE AGES AND PORTIONS**



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**FACULTY OF SCIENCE AND NATURAL  
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UNIVERSITI MALAYSIA SABAH  
2014**

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**THESIS SUBMITTED IN FULFILLMENT FOR  
THE DEGREE MASTER OF SCIENCE**

**FACULTY OF SCIENCE AND NATURAL  
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UNIVERSITI MALAYSIA SABAH  
2014**

## DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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## CERTIFICATION

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## ABSTRACT

*Cassia fistula* Linn. (Golden Shower Tree; Local name: *Rajah Kayu*) is a medium-sized tree belongs to the family Leguminosae. Its different plant portions are used in traditional treatment of various diseases and common ailments in different parts of the world since time immemorial, however still lesser known in Malaysia. In this project, methanolic extracts from different portions (bark, stem, leaf and root) of different age classes, namely class 1 (2-3 years), class 2 (5-10 years) and class 3 (10-15 years) of *C. fistula* were studied for their total extractives yield (TE) and Stiasny Number (SN) were analyzed. The antioxidant activity (AA), total phenolic content (TPC), and total tannin content (TTC) of plant extracts were analysed using 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) Free Radical Scavenging Method and Folin-Ciocalteu Method respectively. Meanwhile, the antifungal activity (AF) of *C. fistula* extracts against 5 types of wood-rotting fungi, namely *Pycnoporus sanguineus* (PS), *Microporus xanthopus* (MX), *Coniophora puteana* (CP), *Trametes* spp. (T) and *Stereum ostrea* (SO) was determined using Poisoned Culture Medium technique. The bark portion of *C. fistula* produced the highest TE (3.24±0.56%) and SN (77.77±0.95%) compared to other portions. Meanwhile, TPC and TTC for *C. fistula* extract ranged from 4.58±0.00% to 21.21±0.05% and 1.18±0.00% to 3.81±0.00% respectively. The bark portion of *C. fistula* showed the highest AA with lowest IC<sub>50</sub> (4.04±0.05 mg/ml – 4.16±0.03 mg/ml) compared to other portions. The AF showed by *C. fistula* extracts against wood rotting fungi was considered low and less effective. The highest inhibition activity obtained in this study was 37.75% (<50% inhibition). *C. fistula* extract was inactive against fungi MX. The overall study showed that, different tree portions displayed greater influences on the chemical characteristic, antioxidant and antifungal activities of *C. fistula* compared with different tree age classes. Bark extracts of *C. fistula* demonstrated a potential as natural tannin-adhesives application. The study reported that, *C. fistula* extracts possessed potential pharmacological properties such as AA and AF against wood rotting fungi. However, further investigation and research on AF of *C. fistula* against wood rotting fungi is recommended in order to identify potential specific compounds and their exact mechanism.

## ABSTRAK

### **CIRI-CIRI KIMIA, ANTIOXIDAN DAN ANTIKULAT AKTIVITI BAGI CASSIA FISTULA LINN. PADA UMUR DAN BAHAGIAN POKOK YANG BERLAINAN**

*Cassia fistula* Linn. atau dikenali nama tempatan Rajah Kayu merupakan pokok bersaiz sederhana dalam keluarga Leguminosae. Pelbagai bahagian pokok ini telah digunakan tujuan rawatan tradisional bagi pelbagai penyakit di serata dunia sejak dahulu lagi. Namun, ia masih kurang dikenali di Malaysia. Ekstrak methanol pokok *C. fistula* dengan bahagian berbeza (kulit, batang, daun, akar) daripada kelas umur berlainan, iaitu kelas 1 (2-3 tahun), kelas 2 (5-10 tahun) dan kelas 3 (10-15 tahun) diujikaji jumlah esktraknya (TE) dan nombor stiasny (SN) telah dianalisis. Aktiviti antioksidan (AA), jumlah kandungan fenolik (TPC) dan jumlah kandungan tanin (TTC) ekstrak tumbuhan telah dianalisis dengan menggunakan Kaedah "1,1-Diphenyl-2-Picryl-hydrazyl" (DPPH) dan Kaedah "Folin-Ciocalteau" masing-masing. Manakala, antikulat aktiviti (AF) bagi 5 jenis kulat kayu reput antaranya *Pycnoporus sanguineus* (PS), *Microporus xanthopus* (MX), *Coniophora puteana* (CP), *Trametes* spp. (T) and *Stereum ostrea* (SO) ditentukan dengan menggunakan teknik Medium Kultur Beracun. Dalam kajian ini, jumlah kandungan ekstrak (TE) ( $3.24 \pm 0.56\%$ ) dan nombor Stiasny (SN) ( $77.77 \pm 0.95\%$ ) didapati paling tinggi di bahagian kulit berbanding dengan bahagian-bahagian lain. Manakala, jumlah kandungan TPC dan TTC *C. fistula* masing-masing adalah di antara  $4.58 \pm 0.00\%$  hingga  $21.21 \pm 0.05\%$  dan  $1.18 \pm 0.00\%$  hingga  $3.81 \pm 0.00\%$ . Bahagian kulit *C. fistula* ekstrak menunjukkan aktiviti antioksidan (AA) tertinggi dengan nilai  $IC_{50}$  yang paling rendah ( $4.04 \pm 0.05$  mg/ml -  $4.16 \pm 0.03$  mg/ml) berbanding dengan bahagian-bahagian lain. *C. fistula* menunjukkan AF yang lemah terhadap jenis kulat kayu yang dikaji dengan kadar perencatan tertinggi  $37.75 \pm 0.00\%$  (<50% perencatan) sahaja. Ekstrak *C. fistula* tidak menunjukkan sebarang AF menentang fungsi MX. Secara keseluruhannya, bahagian pokok lebih mempengaruhi ciri-ciri kimia, antioksidan dan antikulat aktiviti bagi *C. fistula* berbanding dengan umur pokok. Ekstrak kulit *C. fistula* menunjukkan potensinya untuk digunakan sebagai pelekat-tanin semulajadi. Kajian ini turut melaporkan ekstrak *C. fistula* memiliki potensi farmakologi seperti aktiviti antioksidan dan antikulat. Namun, siasatan dan kajian lanjut adalah disyorkan untuk *C. fistula* dalam menentang kulat kayu reput dengan tujuan mengenalpasti potensi sebatian tertentu dan mekanisme yang tepat.

## TABLES OF CONTENTS

	Page
<b>TITLE</b>	i
<b>DECLARATION</b>	ii
<b>CERTIFICATION</b>	iii
<b>ACKNOWLEDGEMENT</b>	iv
<b>ABSTRACT</b>	v
<b><i>ABSTRAK</i></b>	vi
<b>TABLE OF CONTENTS</b>	vii
<b>LIST OF TABLES</b>	ix
<b>LIST OF FIGURES</b>	xi
<b>LIST OF ABBREVIATIONS</b>	xii
<b>CHAPTER 1: INTRODUCTION</b>	
1.1 Background and Purpose of Study	1
1.2 Justification	4
1.3 Objectives	5
<b>CHAPTER 2: LITERATURE REVIEW</b>	
2.1 <i>Cassia</i> : Origin and Distribution	6
2.2 <i>Cassia fistula</i> Linn.	6
2.3 Extractives in Plant	10
2.3.1 Extractives in different tree ages and portions	11
2.4 Phenolic Compounds in <i>C. fistula</i>	17
2.5 Tannins	21
2.5.1 Tannins and Stiasny Number	23
2.6 Methanol Solvent Extraction	24
2.7 Antioxidant Activity	24
2.8 Fungi	27
2.8.1 <i>Pycnoporus sanguineus</i>	28
2.8.2 <i>Microporus xanthopus</i>	29
2.8.3 <i>Coniophora puteana</i>	30
2.8.4 <i>Trametes</i> spp.	31
2.8.5 <i>Stereum ostrea</i>	32



### **CHAPTER 3: MATERIALS AND METHODS**

3.1	Wood Collection and Processing	33
3.2	Methanol Extraction and Drying	35
3.3	Stiasny Number Test	36
3.4	Total Phenol Content	37
3.5	Total Tannin Content	38
3.6	Antioxidant Activity	39
3.7	Antifungal Activity	40
3.8	Statistical Analysis	42

### **CHAPTER 4: RESULTS AND DISCUSSIONS**

4.1	Total Extractives (TE)	43
4.2	Stiasny Number (SN)	46
4.3	Total Phenolic Content (TPC)	48
4.4	Total Tannin Content (TTC)	51
4.5	Antioxidant Activity (AA)	53
4.6	Antifungal Activity (AF)	56

### **CHAPTER 5: CONCLUSIONS AND RECCOMENDATIONS**

5.1	Conclusions	61
5.2	Recommendations	63

### **REFERENCES**

		64
APPENDIX A:	Total Extractives (TE)	75
APPENDIX B:	Stiasny Number (%) (SN)	76
APPENDIX C:	Total Phenolic Content (TPC) (Absorbances)	77
APPENDIX D:	Total Tannin Content (TTC) (Absorbances)	78
APPENDIX E:	Antioxidant Activity (AA) (Absorbances)	79
APPENDIX F:	Antifungal Activity (AF)	80

## LIST OF TABLES

	Page
2.1 Properties and Uses of <i>C. fistula</i> plant parts	9
2.2 Extractives yield (%) of methanol bark extracts of commercial Malaysian Timber	13
2.3 Extractives yield (%) of methanol stem (sapwood and heartwood) extracts of hardwood species of Malaysian Timber	14
2.4 Total Phenolic Content (%) of methanol extracts from bark and stem(sapwood and heartwood) of some Malaysian Timber species	18
2.5 Secondary metabolites in <i>C. fistula</i> plant parts	19
2.6 Yield of tannin contents of different species of medicinal plant from different plant parts	22
4.1 TE (%) (Bark and Stem) obtained from <i>C. fistula</i> trees at different age classes	43
4.2 TE (%) (Bark and Stem) obtained from <i>C. fistula</i> trees at different age classes	44
4.3 SN (%) reaction obtained from <i>C. fistula</i> trees of different age classes at bark and stem portions	46
4.4 SN (%) reaction obtained from <i>C. fistula</i> trees of different age classes at leaf and root portions	47
4.5 TPC (%) of <i>C. fistula</i> plant extracts at three different age classes for bark and stem portions	48
4.6 TPC (%) of <i>C. fistula</i> plant extracts at three different age classes for leaf and root portions	49
4.7 TTC (%) of bark and stem portions at different age class	52
4.8 TTC (%) of leaf and root portions at different age class	52
4.9 IC <sub>50</sub> (mg/ml) of bark and stem portions of <i>C. fistula</i> at different age classes	54

4.10	IC <sub>50</sub> (mg/ml) of leaf and root portions of <i>C. fistula</i> at different age classes	54
4.11	Percentage of Inhibition (%) of <i>C. fistula</i> plant extract in different age classes at bark and stem portions	57
4.12	Percentage of Inhibition (%) of <i>C. fistula</i> plant extract in different age classes at leaf and root portions	58



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## LIST OF FIGURES

	Page
2.1 <i>C. fistula</i> Tree	7
2.2 Structure of some phytoconstituents isolated from <i>C. fistula</i>	20
2.3 Brown rot (upper) and white rot (lower) in wood	28
2.4 <i>Pycnoporus sanguineus</i>	29
2.5 <i>Microporus xanthopus</i>	30
2.6 <i>Coniophora puteana</i>	31
2.7 <i>Trametes</i> spp.	32
2.8 <i>Stereum ostrea</i>	32
3.1 Experimental Design of <i>C. fistula</i> at different age classes, portions and their application	34
3.2 Fine sample powder for different portions	35
3.3 Precipitate (Insoluble phlobaphenes)	37
3.4 Measurement of Fungal Radial Growth	41

## LIST OF ABBREVIATIONS

<b>AA</b>	Antioxidant Activity
<b>AF</b>	Antifungal Activity
<b>BHT</b>	Butylated Hydroxy Toluene
<b>DPPH</b>	Diphenylpicryl-hydrazyl
<b>GAE</b>	Gallic Acid Equivalent
<b>HCl</b>	Hydrochloric Acid
<b>NaCO<sub>3</sub>10 H<sub>2</sub>O</b>	Sodium Carbonate Solutions
<b>PA</b>	Proanthocyanidins
<b>PDA</b>	Potato Dextrose Agar
<b>PVPP</b>	Polyvinylpolypyrrolidone
<b>SN</b>	Stiasny Number
<b>TA</b>	Tannic Acid
<b>TAE</b>	Tannic Acid Equivalent
<b>TPC</b>	Total Phenolic Content
<b>TTC</b>	Total Tannin Content

# CHAPTER 1

## INTRODUCTION

### 1.1 Background and Purpose of Study

Wood extractives are small amount chemical constituents present in wood which are found to be higher concentration in bark of most woods (Helm, 2000). The amounts of extractives in wood can range from 1-20% depending upon species and position within the tree (Walker, 2006). Although their quantities are low, but they play a vital role in protecting the plants against environment pressure, invasion of pathogen, fungi, insects as well as enhance and control the plant growth (Helm, 2000).

Extractives in wood can be solubilized extracted by organic solvents, both polar and non-polar solvents (Walker, 2006). According to Walker (2006), extractives can functions as antifeedants, antioxidants, antivirals, bacteriacides, fungicides, etc. Nowadays, the use of plant, and plant extract or plant-derived chemicals to treat diseases and for other purposes has gained popularity. More than 80% of world's population still relies on plant-based traditional medicines for their primary healthcare needs (Mbwambo *et al.*, 2007). It is estimated that more than 25% of the modern medicines are directly and indirectly derived from plants (Danish *et al.*, 2011). Studies reveal that, present of various polyphenolic compounds, in the plant from family Leguminosae are quite common. Those polyphenolic compounds possess remarkable pharmacological activities (Siddhuraju *et al.*, 2002). *Cassia fistula* Linn. (commonly known as Indian Laburnum or Golden Shower) belongs to the family Leguminosae. These fast growing medium-sized ornamental trees can grow widely in both tropical and subtropical areas, such as India, Sri Lanka, Mauritius, South Africa, Mexico, Brazil, China, Thailand, including Malaysia (Sangetha *et al.*, 2008; Vasudevan *et al.*, 2009).

Studies show that, *C. fistula* is rich with phenolic compounds, such as tannins, flavonoids, and glycosides. (Danish *et al.*, 2011). *C. fistula* is well-known for both medicinal properties and therapeutic uses in many parts of the world since a long time ago, however lesser known in Malaysia. In Malaysia, *C. fistula* is also known as *Rajah Kayu*, *Bereksa* or *Tengguli* (Padua *et al.*, 1999), and they are distributed throughout Malaysia (Sangetha *et al.*, 2008).

*C. fistula* different plant portions have been used in traditional treatment of various diseases and common ailments in different parts of the world since time immemorial (Rizvi *et al.*, 2009). Extractives containing phenolic compounds, such as tannins, occur in nearly every plant, in all climates at almost any portions of the plant (Cannas, 2013). However, amount of those extractives are quite variable depending upon species, position within species, climate, soil condition, etc. (Walker, 2006). Therefore, different portions (bark, stem, leaf and root) from different age classes, namely class 1 (2-3 years), class 2 (5-10 years) and class 3 (10-15 years) of *C. fistula* were studied for their extractives content.

Besides, extractives like tannins occur in almost any part of nearly very plant. Typically, they are concentrated in bark layer where it forms a barrier against micro-organisms (Helm, 2000; Antwi-Boasiako and Animapauh, 2012). They not only act as a defensive compounds in plant at the same time give rise to the wood durability but many of it is also an important ingredient in tannins leather processing and wood adhesives applications. Tannins from eastern hemlock, chestnut oak, and tan oak barks for examples are used in tannins leather processing whereas tannins from those western hemlock, Douglas-fir and redwood barks are used in wood adhesives application (Antwi-Boasiako and Animapauh, 2012). Tannins from *Acacia* spp., *Pinus* spp., quebracho, chestnut, etc. have been highly investigated for production of wood adhesives commercially, but there is no detail study of *C. fistula* tannins extracts as wood adhesives application. Previous study reported that, *C. fistula* is very rich with tannins compounds, especially their bark (Ledwani and Singh, 2006).

Thus, these have generated interest to further study on *C. fistula* tannin extracts as wood adhesives application based on Stiasny Number (SN) test in search for environmental-friendly and renewable sources of tannins. In this study, although there is no detail study of *C. fistula* tannin extract as wood adhesives for wood composites applications, but at least their reactivity towards formaldehyde was determined using SN test.

Furthermore, according to Daniel (2006), different portions of *C. fistula* were proved to possess different medicinal values. Over the past few years, wide range of studies has been done on *C. fistula* plant pharmacological properties, but there is no detail study on antioxidants activity (AA), total phenolic content (TPC), and total tannin content (TTC) of *C. fistula* extracts at different tree ages and portions. Besides, nowadays natural occurring antioxidants from medicinal plants and herbs has gain popularity and greater demand compare to synthetic one. Thus, in this study, antioxidant potentials, phenolic content and tannin content in *C. fistula* from different age classes and portions were determined.

Nowadays, the issue about maintain and preserve wood have gained popularity. As we have observed, the wood itself is susceptible to various harmful agents, for example fungi, bacteria, woodlice, etc. (Schmidt, 2006). Fungi such as blue-stain fungi and red-streaking fungi can cause discolouration of wood, whereas brown rot and white rot fungi contribute to wood decay. Those pathogenic fungi can lead to reduce tree growth and to lessen the wood quality and durability. Therefore, a bulk of investigation has been done to protect and to preserve wood from undergoing deterioration by fungi, for example through organizational protection, natural methods (used of durable wood species), chemical preservation, wood modification, etc. (Goodell *et al.*, 2003). Traditionally, wood preservatives are highly effective against wood decay fungi, but have been restricted due to their toxicity towards an environment and health in long term effect.



Therefore, investigation and development of environment-friendly wood preservatives undoubtedly needed. Previous studies has shown that, *C. fistula* extracts possessed some antifungal properties, antibacterial, anti-inflammatory activities. The plant extracts is also recommended as pest control agents (Dave and Ledwani, 2012). Therefore, in this study different tree ages and portions of *C. fistula* extracts were also tested against various species of wood rotting fungi, namely *Pycnoporus sanguineus*, *Microporus xanthopus*, *Coniophora puteana*, *Trametes* spp. and *Stereum ostrea* .

## 1.2 Justification

*C. fistula* (Indian Laburnum) is a fast growing, medium sized ornamental trees which can cultivate worldwide throughout the tropics, including China, West Indies, Mauritius, India, Sri Lanka, South and East Africa, Mexico, Brazil and even in Malaysia (Bahorun *et al.*, 2005; Sangetha *et al.*, 2008). *C. fistula* is well-known for both medicinal and industrial values in various parts of the world since a long time ago however lesser known in Malaysia. *C. fistula* different plant portions are known to be an important sources of secondary metabolites, notably phenolic compounds (Bahorun *et al.*, 2005). Further, *C. fistula* also known as rich sources of tannins, flavonoids and glycosides (Danish *et al.*, 2011). Extractives, or secondary metabolites in plant, such as tannins occur nearly in every plant, in all climates and can be found almost in any parts of the plants, which also include *C. fistula*. However, amount of those extractives are quite variable depending upon species, position within trees, site specific, etc. (Walker, 2006). Thus, different tree ages and portions of *C. fistula* were used to study for their total extractives content, in order to find out which tree ages or portions can yield most promising quantities of extractives compounds and suitable to undergo harvesting.

In addition, extractives like tannins can widely distributed in nearly every plant, at almost every portions of plants, like stem, bark, root, leaf, fruit, seed, flower and even pods.

*C. fistula* different portions has proved to be important sources of phenolic compounds and their stem bark is especially rich in tannins (Ledwani and Singh, 2006). Thus, in this study tannin extracts from different tree ages and portions of *C. fistula* were used to test on their reactivity towards formaldehyde based on Stiasny Number (SN) test in order to determine their possibility to use in wood adhesives application.

Furthermore, according to Walker (2006), extractives can function as antifeedants, antioxidants, antivirals, bacteriacides, cytotoxins, fungicides etc. *C. fistula* extract are well known for various pharmacological effects, such as antifungal, antioxidant, antibacterial, etc. (Bahorun *et al.*, 2005; Rizvi *et al.*, 2009). Traditional medicinal properties of *C. fistula* and its phytochemical investigation prove its importance as a valuable medicinal plant. In view of above, antioxidant potentials, phenolic content and tannin content in *C. fistula* extracts from different tree ages and portions were determined. Nowadays, preserve and protect wood from damage caused by wood rotting fungi have gained popularity. Some traditional widely used wood preservatives, such as chromated copper arsenate (CCA) have negative impact on environment (Wang *et al.*, 2011). Many scientists studied on wood extractives and their bioactivities try to discover their possible antifungal activities. Therefore, *C. fistula* different tree ages and portions extracts were studied on antifungal activities against 5 types of wood rotting fungi and understand the relationship between the constituents of the extractives and their antifungal activities.

### **1.3 Objective**

The objectives of this study were:

1. To determine the chemical characteristic (total extractives, phenolic and tannin content, stiasny number) of *C. fistula* extracts at different tree ages and portions;
2. To determine the antioxidant and antifungal activities of *C. fistula* extracts at different tree ages and portion

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 *Cassia* : Origin and Distribution

The genus *Cassia* Linn., is a large tropical genus, consists of about 600 species of herbs, shrubs, and trees in the family Leguminosae. (Dave and Ledwani, 2012). *Cassia* species occur in a range of climates. They are widely distributed throughout Asia, including India, Mauritius, China, East Africa, South Africa, America, Mexico, West Indies and Brazil (Mazumder *et al.*, 2008). There are hundreds of species of *Cassia* which occurs more than 1000 names. Some important species are *Cassia fistula*, *Cassia grandis*, *Cassia hirsutica*, *Cassia alata*, *Cassia tora*, *Cassia auriculata*, etc (Ayol *et al.*, 2007). Most of the plants of the genus are well-known in Indian system of medicine for their cathartic, purgative and antibiotic properties. *Cassia* species are reported to contain alkaloids, sitosterols, anthraquinone glycosides, tannins and flavonoids (Mazumder *et al.*, 2008).

#### 2.2 *Cassia fistula* Linn.

*C. fistula* Linn. (commonly known as Indian Laburnum or Golden Shower Tree) is a medicinal plant whose one or more of its parts like bark, stem, root, leaf, etc. contains certain chemical constituents which have both medicinal and industrial values. For the past few decades, there has been an increasing interest in the investigation of different extracts from these traditional medicinal plants, as potential sources of therapeutic agents (Mazumder *et al.*, 2008). Native to India, the Amazon and Sri Lanka, *C. fistula* has become extensively distributed throughout various countries, including India, Mauritius, China, East Africa, South Africa, America, Mexico, West India and Brazil (Bahorun *et al.*, 2005; Vasudevan *et al.*, 2009).

Malaysia, is a country rich with plant biodiversity. *C. fistula* (Figure 2.1) is widely grow as an ornamental tree with beautiful bunches of yellow flowers (Bahorun *et al*, 2005), and they are distributed throughout Malaysia (Sangetha *et al.*, 2008). *C. fistula* is used as an ornamental and shade tree around houses; on the edges of road; and in the streets, parks, and gardens of towns.



**Figure 2.1: *C. fistula* Tree (Shantha, 2011).**

*C. fistula* is a medium-sized deciduous tree, can grow up to 10-20 m tall with fast growth rate (Sangetha *et al.*, 2008). In Malaysia, they are called as *Rajah Kayu*, *Bereksa* or *Tengguli* (Padua *et al.*, 1999). They produce yellow golden flower and hang in showering bunches of up to 40 cm long, earning its common name of "Golden Shower Tree" (Taylor, 1996; Neelam *et al.*, 2011). This plant has compound leaves, with 4-8 pairs of opposite leaflet. The bark of this plant has greenish grey colour and smooth when young but turns rough and dark brown when old (Warrier, 1994; Neelam *et al.*, 2011). *C. fistula* wood is hard, heavy, strong and durable with specific gravity of 0.9, thus suitable for cabinet work,

interior work, carts, fence posts, wheels and mortar, agricultural implements, constructions, etc. (Michael, 1999; Selvam, 2007). Besides, *C. fistula* wood also use for producing excellent charcoal due to high durability (Manandhar, 2002). Additionally, the bark of *C. fistula* is also used for tannin extraction. In India and Java, the bark of *C. fistula* is used for tanning and dyeing (Prasad and Reshmi, 2007; Kumar, 2009). In Myanmar, tannin for leather industry is in great demand. *C. fistula*, has been considered as one of the important sources of trees in Myanmar others than *Acacia leucopholea*, mangrove species that are yielding tannin (Khin, 1995). Recent studies by Hanif *et al.* (2006) reported that *C. fistula* biomass like their leaves, branches, stem bark and pods bark can be reused as biomaterial for removal of pollutant especially Ni (II) under aqueous solutions.

Further, according to Warriar (1994), the whole part of *C. fistula* tree can be applied for therapeutic purposes. These were also reported by Daniel (2006), all parts of the tree include bark, stem, root, fruit, seed and even pod possesses their own medicinal value. Traditionally, the bark and the wood are used to fight against dysentery, treating leprosy, jaundice, syphilis and cardiac ailments (Prasad and Reshmi, 2007; Sangetha *et al.*, 2008). The bark is laxative, anthelmintic, emetic, febrifuge, diuretic and depurative, and useful in boils, pustules, leprosy, ringworm, colic, dyspepsia, fever, diabetes, etc. (Vaidyaratnam, 1994). Meanwhile, a decoction of root or root bark is recommended for cleansing deep and featering wound (Sangetha *et al.*, 2008) and used for backward fever (Danish *et al.*, 2011). The roots are astringent, cooling purgative, febrifuge, and tonic (Vaidyaratnam, 1994), and are also useful in rheumatism, hemorrhages, ulcers, skin diseases and leprosy (Daniel, 2006). The leaves of *C. fistula* are laxatives, antiperiodic and used in rheumatism (Bhakta *et al.*, 1999; Daniel, 2006). The properties and uses of different parts of *C. fistula* are summerized in Table 2.1.

**Table 2.1: Properties and Uses of *C. fistula* plant parts**

<b>Plant Part</b>	<b>Properties</b>	<b>Uses</b>
<b>Bark and Stem</b> (Vaidyaratnam, 1994; Daniel, 2006; Prasad & Reshmi, 2007)	laxative, tonic, anthelmintic, emetic, febrifuge, diurectic, depurative	leprosy, dysentery, jaundice, syphilis, boils, pustules, ring worm, colic, dyspepsia, constipation, fever, diabetes, strangury, heart diseases
<b>Root and root bark</b> (Vaidyaratnam, 1994; Ali <i>et al.</i> , 2004; Daniel, 2006; Sangetha <i>et al.</i> , 2008; Danish <i>et al.</i> , 2011)	astringent, cooling, strong purgative, febrifuge, tonic	skin diseases, rheumatism, hemorrhages, ulcers, leprosy, syphilis, burning sensation, fever, chest pain, joint pain, migraine, heart diseases
<b>Leaves</b> (Vaidyaratnam, 1994; Bhakta <i>et al.</i> , 1999; Danish <i>et al.</i> , 2011)	laxative, purgative, antiperiodic, depurative	ulcer healing, rheumatism ringworm infections, cough, jaundice, piles, external skin eruptions, eczema
<b>Flower</b> (Vaidyaratnam, 1994; Danish <i>et al.</i> , 2011)	bitter, acrid, cooling, emollient, expectorant, demulcent, purgative, febrifugal, anti-bilious	burning sensation, dry cough and bronchitis, skin diseases, pruritus
<b>Fruit and Pulp</b> (Vaidyaratnam, 1994; Daniel, 2006; Prasad & Reshmi, 2007)	purgative, analgesic, antipyretic (against malaria), cooling, emollient, laxative	snake bites, anthrax, dysentery, leprosy, diabetes, biliousness, skin diseases, inflammations, flatulence, rheumatism
<b>Pod</b> (Daniel, 2006; Sakulpanich and Gritsanapan, 2009; Danish <i>et al.</i> , 2011)	laxative, purgative, astringent	thoracic obstructions, heat of blood, gout, rheumatism
<b>Seed</b> (Daniel, 2006; Danish <i>et al.</i> , 2011)	laxative, carminative, antipyretic, emetic, cathartic	ameobiasis, constipation, jaundice, biliousness, skin diseases, swollen throat



### 2.3 Extractives in Plant

Extractives are the small amount of chemical constituents present in wood that can be solubilized (extracted) by organic solvents. According to Hon and Shiraishi (2000) and Anon (2000), extractives can be gained or removed from wood by extraction with inert neutral solvents such as water, alcohol, acetone, benzene and ether. Extractives are found in higher concentrations in the bark of most woods and they play a role in protecting the plants against environment stress, invasion of pathogens as well as enhance the plant growth (Helm, 2000). They are not part of the wood structure like cellulose, hemicellulose, and lignin, but they do contribute to such properties, like color, odor taste, decay resistance, strength, hygroscopicity, density and flammability (Anon, 2000).

Hon and Shiraishi (2000), has classified extractives as secondary metabolites due to the factor that the distribution of certain chemical component restricted only to certain species. Extractives, known as secondary metabolites, secondary products or natural products because they have no direct roles in the processes of photosynthesis, respiration, solute transport, translocation, protein synthesis, nutrient assimilation and differentiation in plant (Hartmann, 1991). Plant secondary metabolites can be divided into three chemically distinct groups, namely terpenes, phenolics, nitrogen and sulphur containing compounds (Mazid *et al.*, 2011). They act as defensive compounds in plants against a variety of herbivores, and pathogenic microorganisms as well as various kinds of abiotic stresses (Mazid *et al.*, 2011).

Extractives or secondary metabolites actually are a group of cell wall chemical which consists of fats, fatty acids, fatty alcohols, phenols and many minor organic compound which exist in term of monomers, dimers and polymers (Rowell, 2005). They are very restricted distribution than primary metabolites. They only found in one plant species or a taxonomically related group of species (Mazid *et al.*, 2011). Hillis (1987) revealed that, not just the types of extractives present in wood varies, so also does the amount. The amount of extractives can vary also according to vertical as well as radial position from which the sample was taken. Sherry (1971) has shown that amount of tannins (polyphenols) and extractives in bark is greatest at the bottom rather than at the top of the tree of *Acacia mearnsii*. According to Hill (2006), level of extractible material in wood can ranging from approximately 0.5% to around 20% by weight depending on the species. Further, Anon (2000) and Walker (2006) revealed that, amounts of extractives in wood depending on such factors, namely tree species, position within the tree, growth conditions and also time of year the tree is cut.

### **2.3.1 Extractives in different tree ages and portions**

Extractives can be isolated from bark and wood stem, as well as from leaves, fruits and roots and they are highly concentrated at bark portions of most woods (Helm, 2000).

#### **a. Bark**

Bark, is make up of complex biomass material together with various chemical constituents, including mainly polysaccharides and lignin and various extractives (Huang and Yan, 2014). They contains a much higher content of extractives that is composed of diverse group of chemicals, example fats, waxes, sterols, terpenes and terpenoids, phenolics, flavones and flavonoids and polyphenols (tannins and polyphenolic acids) compared with wood stem (Huang and Yan, 2014). These extractives or secondary metabolites have shown their potentials in antioxidant, antifeedants, bacteriacides properties (Walker, 2006). Research and development have shown the potentials of these various types of chemical compounds present in bark extractives, thus could be utilized in different industrial fields such as, wood