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ANTIFUNGAL ACTIVITY OF CASSIA FISTULA L. EXTRACTS AT DIFFERENT PORTIONS (BARK, STEM, ROOT, LEAF) AND AGE CLASSES

SUMMARY

Fungi deterioration of wood is the most common damaging problems of timber industries worldwide. Most wood, especially less durable wood is prone to attack by a wide-range of fungi; thereby a broad spectrum of control is undoubtedly necessary. In this study, the antifungal activity of methanolic extracts of bark, stem, leaf and root of *Cassia fistula* L. under different age classes, namely Class A (2-3 years), Class B (5-10 years), and Class C (10-15 years) against both white-rot and brown-rot fungi were investigated. The fungi used were *Pycnoporos sanguineus*, *Microporus xanthopus*, *Coniophora puteana*, *Trametes* species and *Stereum ostrea*. Generally, methanolic extracts of *Cassia fistula* L. at different portions and age classes exhibited good and selective antifungal activity against most of the fungi tested, except fungi *Microporus xanthopus*.

Key words: Antifungal, Age, *Cassia fistula* L., Portions, Wood rotting fungi

INTRODUCTION

The plant produces a diverse amount of secondary metabolites, namely phenolic compounds. These secondary metabolites, such as polyphenols play vital roles in defense against plant diseases cause by pathogens, harmful pest and others microorganism. There are several properties that are shown by these phenolic compounds, such as antifungal (Castillo et al., 2010), antimicrobial (Pengelly, 2004), bactericidal, antiseptic, etc. *Cassia fistula* L. also known as Golden Shower or *Rajah Kayu* in Malays belongs to the family Leguminoceae able to grow widely in both tropical and sub-tropical areas, including Malaysia (Sangetha et al., 2008). Extensive study has been done during the past few decades on various parts of *Cassia fistula* L. and it already established fact that *Cassia fistula* L. plant parts are a great important sources of secondary metabolites, notably phenolic compounds (Bahorun et al., 2005; Duraipandiyan and Ignacimuthu, 2007). All the plant parts of *Cassia fistula* L. have been studied extensively and thoroughly previously, and reported its antifungal, antimicrobial (Ali et al., 2004; Phongpaichit et al., 2004), anti-inflammatory (Ilavarasan et al., 2005), antibacterial and many other pharmacological activity.

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Wood, especially less durable wood is prone to fungal wood decay, infection by wood fungi such as white rot and brown rot reduced both durability and quality of wood itself. Failure of control might results serious economic and resources losses. Wood preserving industry used synthetic preservatives in order to control wood decay and deteriorations cause by fungi, but that pose negative impact towards human health and environment as well make it unsuitable to be used in long term (Astiti and Suprapta, 2012). Therefore, investigations of various plants produce preservatives agents which are environmental friendly and safe to human health is undoubtedly necessary.

Many studies have reported the antipyretic, antifungal, antibacterial, anti-inflammatory (Kumar et al., 2006) of *Cassia fistula* L. and even the plant extract is also recommended as pest control agents. However, there is less report on antifungal activity shown by *Cassia fistula* L. extracts of different portions (bark, stem, leaf and root) at different age classes against wood rotting fungi (white rot and brown rot). Thus, this paper addressed the antifungal activity shown by *Cassia fistula*. L. methanolic extracts at different portions (bark, stem, leaf, root) and age classes against wood rotting fungi.

MATERIAL AND METHODS

Sample Collection and Processing

Total of nine *Cassia fistula* L. trees were collected from Likas Flora Nursery, Telipok. Therefore, three trees were sampled from each age classes, namely Class A (2-3 years), Class B (5-10 years), and Class C (10-15 years). All the wood samples were divided manually into four portions (bark, stem, root and leaf) accordingly. Whereas, for stem and bark portion, they were further divided into another three sub-portions namely; bottom (B), middle (M) and top (T) for all the nine trees and labeled properly. All wood samples were washed and went for air dried for 2 weeks before chipped and grinded into powder form around 500 µm. The powders were packed and stored in dry area to avoid humidity and fungus for further study.

Sample Extraction and Drying

The samples (50 g) powder were undergone extraction with 70% of methanol on ratio 1:6 w/v at 75^0 C for 3 hours based on Hoong et al. (2009) and Hoong et al. (2010) method. The crude extracts were then filtered using Whatmans paper No.1 (150 nm). The filtered extracts (liquid form) were further dried manually using waterbath (55-60°C) in order to remove methanol solvent to form dry extracts (solid form) and those dry extracts were further dry in an oven at 50°C until weight was constant. After that, labeled and stored those dry extractives properly until further use.

Evaluation of Antifungal Activities

All *Cassia fistula* L. extracts for different portions (bark, stem, leaf, root) and age classes were subjected to antifungal activity assays using fungi

Pcynoporos sanguineus, Microporus xanthopus, Coniophora puteana, Trametes species, *Stereum ostrea* which were obtained from Forest Research Institute Malaysia (FRIM) Kepong, Selangor.

The antifungal assays method used was based on Satish et al. (2007) with modification. For sample treatment, 1 ml of diluted *Cassia fistula* L. extracts plus 19 ml of PDA (Potato Dextrose Agar) were poured into each petri dish, mixed thoroughly and allowed to solidify. PDA medium without the plant extracts but substitute with 1 ml distilled water was served as control. Disc of 0.5 cm culture of the test fungi was placed at the center of the petri dish (both sample and control) and incubated at 27^{o}C inside incubator for 5 days. The efficacy of each plant extracts at different age classes of *Cassia fistula* L. were evaluated by measuring fungal radial growth (cm) using ruler. The antifungal activity in terms of percentage inhibition was calculated by using formula below:

$$\% \text{ Inhibition} = (C-T)/C \times 100$$

where C= average increase in mycelial growth in control, T= average increase in mycelial growth in treatment

Statistical Analysis

Data analysis of antifungal activity of different extracts of *Cassia fistula* L. at different age classes against the test fungi were performed using correlation test in order to identify if whether or not significant differences exist.

RESULTS AND DISCUSSION

Table 1 and 2 showed the results of bark, stem, leaf and root extracts at different age classes of *Cassia fistula* L. which exhibited selectively antifungal activity against fungi tested. Generally, inhibition activity of *Cassia fistula* L. plant extracts at different portions against fungi tested increase from age Class A to age Class C. Based on the result, stem, bark, and root extracts showed remarkable inhibition activity against *Stereum ostrea*, *Coniophora puteana* and *Pcynoporos sanguineus* from age Class A to C, especially age Class C (10-15 years). However, bark extract showed slightly lower inhibition activity against *Pcynoporos sanguineus*, as compared to stem and root extracts. Meanwhile, leaf extract showed only good inhibition activity against *Pcynoporos sanguineus*, but less towards others types of fungi. Moreover, both stem bottom (B) portions and bark bottom portion (B) extracts seen to show generally higher antifungal activity against fungi tested compared to middle (M) and top (T) portions from age Class A to C.

Stem extract from age Class C, showed remarkable inhibition activity towards *Coniophora puteana* (37.02%), and followed by *Stereum ostrea* (35.21%), *Pcynoporos sanguineus* (28.40%), *Trametes* species (13.97%) and no inhibition activity against *Microporus xanthopus* (- 8.00 %). Whereas, bark extract from age Class C showed high but lower inhibition activity compared with stem extract against *Stereum ostrea* (34.79%) followed by *Coniophora puteana* (29.15%), *Pcynoporos sanguineus* (9.85%), *Trametes* species (3.22%), and again no inhibition activity against *Microporus xanthopus* (-3.00%). For root extract, highest inhibition activity also been observed in age Class C similar to both stem and bark extracts. But interestingly, root extract showed high inhibition activity against *Stereum ostrea* (37.50%) better than both stem and bark extracts, followed by *Coniophora puteana* (27.26%), *Pcynoporos sanguineus* (20.32%) much better than bark extract, *Trametes* species (17.20%) again much better than both stem and bark extracts, and finally no inhibition activity towards *Microporus xanthopus* (-3.00%). Amazingly, leaf portion at age class C showed good inhibition activity towards *Pcynoporos sanguineus* (18.01%) which was 8.16% higher than bark extract however much lower as compared to root and stem extracts (Table 2).

From the study, all plant extracts from different age classes showed some inhibitory activity against *Trametes* species, except for leaf extract. Unfortunately, there was no inhibition activity showed for all plant extracts (bark, stem leaf and root) against fungi *Microporus xanthopus*. Statistical analyses showed that there were significant differences between tree portions (stem, bark, leaf, root) and age classes with all fungi tested, except *Coniophora puteana*. Tree portions showed significant differences with *Pcynoporos sanguineus* ($r=0.587$, $p \leq 0.01$), *Stereum ostrea* ($r=-0.382$, $p \leq 0.01$) and *Trametes* species ($r=0.312$, $p \leq 0.01$). Meanwhile, different age classes showed significant differences with *Stereum ostrea* ($r=0.406$, $p \leq 0.01$) and *Microporus xanthopus* ($r=0.233$, $p \leq 0.05$). However, there was no significant difference between tree portion and age classes with *Coniophora puteana*. Thus, different tree portions and age classes might probably responsible to the variability of antifungal activity showed by different fungi tested in this study.

Based on Bahorun et al. (2005), *Cassia fistula* L. plant parts are well-known for their secondary metabolites, namely phenolic compounds. During the past few decades, impressive amounts of chemical constituents have been isolated from various parts of *Cassia fistula* L. and their characteristic have been studied thoroughly (Rizvi et al., 2009). Besides, *Cassia fistula* L. has also been reported for its antifungal, antimicrobial, antioxidants, antibacterial, anti-inflammatory and many others medicinal properties as well. Moreover, according to Dave and Ledwani, (2012), there were abundant of bioactive polyphenolics in *Cassia fistula* L. bark, stem, leaf and root extracts that have been reported such as proanthocyanidins (condensend tannins), flavonoids, catechins, alkaloids, antraquinones, anthraquinones derivatives, triterpene derivatives, etc. Therefore, those bioactive compounds might act individually or synergy contributes to the inhibition effect showed by different portion extracts in this study.

Table 1: Antifungal activity (%) of Cassia fistula plant extract of different age classes at bark and stem portions

		Antifungal Activity (%)				
Age Class	Portion	*Pcynoporos sanguineus*	*Microporus xanthopus*	*Coniophora puteana*	*Trametes* sp.	*Stereum ostrea*
A	Bark (B)	7.62	-10.00	27.81	1.51	34.38
	Bark(M)	7.16	-7.00	26.70	1.72	31.25
	Bark (T)	6.69	-5.00	26.33	1.29	33.75
		(7.16)	**(-7.33)**	**(26.95)**	**(1.51)**	**(30.13)**
	Stem (B)	24.94	-6.00	29.65	10.11	17.50
	Stem(M)	21.48	-8.00	29.65	8.60	12.50
	Stem (T)	27.71	-7.00	29.47	7.10	14.38
		(24.71)	**(-7.00)**	**(29.59)**	**(8.60)**	**(14.79)**
B	Bark(B)	7.16	-2.00	34.25	2.78	31.25
	Bark(M)	6.70	-5.00	32.78	2.58	32.50
	Bark (T)	6.92	-6.00	24.68	2.37	34.38
		(6.93)	**(-4.30)**	**(30.57)**	**(2.58)**	**(32.71)**
	Stem (B)	22.63	-3.00	34.25	13.10	28.13
	Stem(M)	26.56	-8.00	35.54	12.47	26.88
	Stem (T)	21.48	-4.00	33.15	10.11	27.50
		(23.56)	**(-5.00)**	**(33.65)**	**(11.90)**	**(27.50)**
C	Bark (B)	11.08	-4.00	26.88	2.79	33.13
	Bark(M)	9.93	-3.00	28.17	3.22	35.00
	Bark (T)	8.54	-2.00	32.41	3.65	36.25
		(9.85)	**(-3.00)**	**(29.15)**	**(3.22)**	**(34.79)**
	Stem (B)	29.56	-6.00	37.75	15.05	37.50
	Stem(M)	28.40	-8.00	37.02	13.97	35.00
	Stem (T)	27.25	-10.00	36.28	12.90	33.13
		(28.40)	**(-8.00)**	**(37.02)**	**(13.97)**	**(35.21)**

Note: 1. Negative value indicate no inhibition activity

2. (B) represent Bottom, (M) represent Middle, (T) represent Top

3. Values in bracket with bold indicate mean

Table 2: Antifungal activity (%) of Cassia fistula plant extract of different age classes at leaf and root portions

		Antifungal Activity (%)				
Age Class	Portion	*Pcynoporos sanguineus*	*Microporus xanthopus*	*Coniophora puteana*	*Trametes* sp.	*Stereum ostrea*
A	Leaf	16.63	-7.00	7.55	-5.81	6.25
	Root	16.63	-2.00	25.78	13.98	33.13
B	Leaf	16.86	-6.00	6.99	-5.37	9.38
	Root	19.17	-2.00	26.34	16.34	34.38
C	Leaf	18.01	-5.00	7.92	-4.30	12.50
	Root	20.32	-3.00	27.26	17.20	37.50

Note: 1. Negative value indicate no inhibition activity

2. (B) represent Bottom, (M) represent Middle, (T) represent Top

Moreover, *Cassia fistula* L. is very rich in tannins, especially their bark. According to Lai and Liew (2013), bark extracts of *Cassia fistula* L. from different age classes showed total means of 16.67% of total phenolic content (TPC) and 3.12 % of total tannin content (TTC). As we know that, tannins are widespread and largely distributed in plant kingdom almost every part in every single tree, plant and herbs. According to Lai and Liew (2013), not only bark of *Cassia fistula* L. but other portions such as stem, root and leaf also served good promising content of tannin compounds as well. Tannins serve as a natural astringent in plant tissue, according to Tatiya et al. (2011), tannins can act as growth inhibitors towards many microorganisms including bacteria, fungi, and yeast. Another earlier study by Onadapo and Owonubi (1993) reviewed that, tannins present at low concentration able to inhibit growth of micro-organism, whereas at high concentration, it can act as antifungal agents by coagulating the protoplasm of the micro-organism. Even though, we cannot clearly justify that tannins are the main chemical constituents which contribute to the inhibition activity shown by different portion extracts in this study, but there is somehow possible and of course further study is undoubtedly needed. Whereas, according to Daniel (2006) and Warrier (1994), and many other researchers confirmed that whole parts of *Cassia fistula* L. possesses their own medicinal values and possible applied for therapeutic purposes. Therefore, there is rational behind the remarkable antifungal activity shown by most wood extracts at different age classes against fungi tested.

Meanwhile, leaf extract showed slightly weak antifungal activity against fungi tested as compared to the rest. This is most probably because types of wood rotting fungi (brown rot and white rot) used in this studied was different with fungi (pathogenic fungi), yeast, bacteria used by others researcher previously. Thus, their antifungal properties might vary as well. Besides, most study discover that, fungi species have different morphological structure than bacteria and yeast due to present of thicker cell walls which contain high percentage of chitin. This chitin present in wood rotting fungi might reduce the antifungal effect showed by different *Cassia fistula* L. part extracts against fungi tested as well.

Interestingly, root extracts of *Cassia fistula* L. at different age classes, especially Class C also showed promising antifungal activity against several fungi such as *Stereum ostrea*, *Coniophora puteana*, *Pcynoporos sanguineus* and *Trametes species* which compatible with both bark and stem extracts. As we know that, root lies below the surfaces of the soil and contain tannin (Lai and Liew, 2013) and the reason of their (tannins) in root tissue probably act as chemical barriers against plant pathogens. Therefore, it is reasonable to assume that, tannin together with others bioactive compounds in root tissue of *Cassia fistula* L. might enhance the possibility high antifungal activity showed in this study.

CONCLUSIONS

From the above investigation, crude extracts obtained from various portions of *Cassia fistula* L. showed selectively antifungal effects towards wood rotting fungi tested. Highest antifungal activity was observed in age Class C (10-15 years), especially for stem, root and bark extracts towards *Coniophora puteana*, *Pcynoporos sanguineus*, *Stereum ostrea*. Although, different age classes doesn't showed much effect on antifungal activity for most of the fungi tested as compared to different tree portions, but their influences should not be ignored as well, further and detail study might need in order to have a clear image on how strong they're actually correlated with each other. Besides, further and more specific studied needs to be done in order to find out possible chemical constituents which corresponding to the antifungal activity of *Cassia fistula* L. extracts, at the same time evaluate the potential effectiveness of the *Cassia fistula* L. extracts as the antifungal agents in future.

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ANTIFUNGALNA AKTIVNOST EKSTRAKTA CASSIA FISTULA L. IZ RAZLIČITIH DJELOVA (KORA, STABLO, KORIJEN, LIST) I KLASA STAROSTI

SAŽETAK

Propadanje drveta izazvano gljivicama je jedno od najčešćih problema sa kojim se susreće drvna industrija širom svijeta. Najveći broj vrsta,posebno manje trajnog drveta, podložno je uticaju velikog broja gljivica,zbog čega je, bez sumnje, potreban širok spektar kontrola. U ovomistraživanju, ispitivana je antifungalna aktivnost metanolskih ekstrakatakore, stabla, lista i korijena Cassia fistula L. iz različitih klasastarosti, a to su Klasa A (2-3 godine), Klasa B (5-10 godina), i Klasa C(10-15 godina) protiv gljivica koje izazivaju i bijelu trulež i smeđu trulež. Vrste gljivica obuhvaćenih ovim istraživanjem bile su vrste Pycnoporos sanguineus, Microporus anthopus, Coniophora puteana, Trametes species i Stereum ostrea. Generalno, metanolski ekstrakti Cassia fistula L. iz različitih djelova i klasa starosti pokazali su dobru i selektivnu antifungalnu aktivnost protiv najvećeg broja ispitivanih gljivica, izuzev Microporus xanthopus.

Ključne riječi: Antifungalni, starost, Cassia fistula L., djelovi, gljivice, trulež drveta