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Anti Tuberculosis Activity of Forest Kedondong (Spondias pinnata) Stembark Extract Against Multiple Drug Resistance (MDR) Strain of Mycobacterium Tuberculosis

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Background: Forest Kedondong (*Spondias pinnata*) traditionally known as "*loloh cemcem*" and commonly used as a chronic cough remedy. Previous research showed that methanol extract of Forest Kedondong leaves active against MDR strain of *Mycobacterium tuberculosis*. The aim of this study were to determine the phytochemical constituent and anti tuberculosis activity of stem bark extract of this plant against MDR strain of *M. tuberculosis*. **Method:** Coarsely powder of Forest Kedondong stem bark was extracted successively with n-hexane, chloroform and 80% ethanol. Anti tuberculosis assay of chloroform and ethanol extract was conducted using proportion method with Lowenstein-Jensen medium within 3 different concentration of extract of 1, 10, and 100 mg/mL. Activity was evaluated by inhibition of extract against *M. tuberculosis* growth, which was calculated by mean reduction in number of colonies on extract containing medium compared to control. **Results and Discussion:** Phytochemical test showed that chloroform extract contains terpenoid and flavonoids. Ethanol extract contains terpenoid, polyphenols and flavonoids. These extracts were active against MDR strain of *M. tuberculosis* with 100% inhibition at concentration of 100 mg/mL. Chloroform extract has higher inhibition against *M. tuberculosis* growth than Ethanol extract. **Conclusions:** These extracts were potentially developed to an anti tuberculosis constituent from natural product.

Keywords: Kedondong stembark, chloroform extract, Ethanol extract, *Mycobacterium tuberculosis,* anti tuberculosis.

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INTRODUCTION

Tuberculosis is infectious disease caused by *Mycobacterium tuberculosis*, which mostly attack lungs. According to WHO report (2013), Indonesia was become the 3rd country with high prevalence of tuberculosis in Asia after India and China. Pandemic of HIV/AIDS significantly increase tuberculosis cases all over the word. Mortality of tuberculosis more frequent in poor and developing countries. Another problem arise in combatting tuberculosis is the presence of resistant strain of *M. tuberculosis*, including mono resistant, multidrug resistant and extended drug resistant strains of *M. tuberculosis*. Treatment of multidrug resistant tuberculosis need higher cost and possibly cause more side effects and drug toxicity. Therefore, many research has been conducting to develop new anti tuberculosis agent.[1]

Medicinal plants have been proven to be an important resource of many therapeutic agents. WHO (2003) also recommended the usage of ethno-medicinal plants in health prevention, promotion and curative of disease. *Spondias pinnata* which is locally known as Forest kedondong, was used as traditional drink in Bali known as "loloh cemcem".[2]

Leaves of this plant also traditionally used for chronic cough remedy. Forest kedondong belongs to Anacardiaceous family. Methanol extract of Forest kedondong leaves showed potent inhibition against multidrug resistant (MDR) strain of *M. tuberculosis in vitro*.[3] N-hexane extract also revealed inhibition against MDR strain of *M. tuberculosis*.[4,5] Extract of *S. pinnata* fruit revealed cytotoxic effect and antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*.[6] Extract of *S. pinnata* stembark also reported for its anthelmintic activity and antioxidant.[7,8] In present research we evaluate anti tuberculosis activity of *S. pinnata* stembark extract against MDR strain of *M. tuberculosis*.

MATERIAL AND METHODS

Plant material

Forest kedondong stembark was collected from Badung, Bali-Indonesia. Identification of plant specimen was conducted at Kebun Raya Eka Karya, Bedugul, Tabanan-Indonesia.

Bacteria *M. tuberculosis* MDR

Bacteria was obtained from Clinical Microbiology Department, Sanglah General Hospital, Denpasar, Bali-Indonesia.

Extraction

Coarsely dried powder of Forest kedondong stembark (50 g) was extracted successively with n-hexane, chloroform and 80% of ethanol to obtain chloroform extract (2.50 g) and ethanoic extract (5.05 g).

Phytochemical test

Phytochemical test was conducted according to methods previously described by Evans et al. (2009), Jones and Kinghom (2006) and Depkes RI (1989) to detect the presence of alkaloids (Dragendorff reagent), saponins (ability to produce stable foam), polyphenols (ferric chloride reagent), steroid, terpenoids and glycosides (Lieberman-Bouchard reagent), flavonoids (with the use of boric acid and oxalic acid).[9-11]

Activity Assay

Anti tuberculosis activity assay was conducted using proportion method, according to Gupta et al. (2010).[12] Extract was added into Lowenstein-Jensen medium to give serial concentration of extract of 1, 10, and 100 mg/mL.

Control group only received 1% dimethyl sulfoxide. Bacteria then inoculated on this medium, incubated in 5% CO_2 incubator, 37^0C for 6 weeks. Observation of colonies growth was done starting from 3rd until 6th week. Inhibition of extract against *M. tuberculosis* was calculated by comparing colonies growth in treatment group which received series concentration of extract to control.

RESULTS AND DISCUSSION

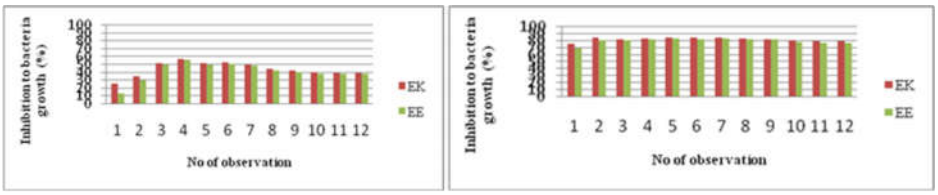
In extraction process, ethanol solvent gave the higher yield than chloroform. N-hexane is used to remove non polar soluble components like resins and other miscellaneous compounds. Phytochemical test showed that chloroform extract contains terpenoids and flavonoids and ethanol extract contain terpenoids, flavonoids and polyphenols. The forming of brown color ring indicated presence of terpenoids and flavonoids were represented by appearance of intensive yellow fluorescence under UV light 366nm. The color changes of ethanoic extract into dark green color indicated the presence of polyphenols.

The result of anti tuberculosis activity assay of extracts was shown in table 1.

Table 1. Inhibition of extract of Forest kedondong stembark against MDR strain of *M. tuberculosis*

Observation		Inhibition against M. tuberculosis growth (%)					
		Extract concentration (mg/mL)					
		Chloroform extract			Ethanoic extract		
		1	10	100	1	10	100
3rd week	1	25.00	75.00	100	12.50	68.75	100
	2	35.29	84.31	100	29.41	80.39	100
	3	51.96	82.35	100	49.02	80.39	100
4th week	4	57.24	83.45	100	55.86	82.07	100
	5	51.15	85.06	100	50.00	83.91	100
	6	52.11	84.39	100	50.24	81.46	100
5th week	7	49.79	84.55	100	48.41	82.83	100
	8	44,17	83.33	100	42.50	82.50	100
	9	41.77	82.33	100	40.56	81.53	100
6th week	10	39.92	80.24	100	38.34	78.66	100
	11	39.39	79.55	100	37.88	77.27	100
	12	39.39	79.55	100	37.88	77.27	100

Observations were made from 3rd to 6th week because this period is growth phase of *M. tuberculosis* on L-J medium.[13] Increasing concentration of chloroform and Ethanol extract on medium resulting in higher inhibition to *M. tuberculosis* growth. Comparison of inhibition of each extracts was shown in figure 1.



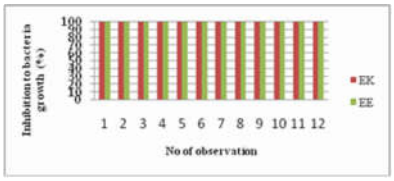


Figure 1. Comparison of inhibition of chloroform (EK) and Ethanol extract (EE) of Forest kedondong stembark against *M. tuberculosis* growth at extract concentration of 1 mg/mL (A), 10 mg/mL (B) and 100 mg/mL (C)

At the extract concentration of 1 and 10 mg/mL, chloroform extract gave higher inhibition than Ethanol extract (Figure 1 (A) and (B)). Treatment with extract at concentration of 100 mg/mL, which is shown in Figure 4.1 (C), chloroform and Ethanol extract of Forest Kedondong stembark able to inhibit growth of *M. tuberculosis* during the observation period by inhibition of 100%. According to Gupta et al. (2010), the extract is stated to have anti tuberculosis activity when inhibition to bacteria growth is ≥90%. Based on these criteria, chloroform and Ethanol extract of Forest kedondong stembark had anti tuberculosis activity against MDR strain of *M. tuberculosis* at concentration of 100 mg/mL.[12]

Various studies on anti tuberculosis activity of flavonoid and terpenoids also been reported. Flavonollaburnetin isolated from Ficuscordata and Ficus*chlamydocarpa* also showed anti tuberculosis activity with MIC value of 4.88 µg/mL (Kuete, 2008). Isoflavonoids isolated from the roots of *Sesbaniagrandiflora* showed anti tuberculosis activity against *M. tuberculosis H37Rv* with MIC of 50 µg/mL (Hasan, et al., 2012). According to Brown et al. (2007), many flavonoids can inhibit fatty acid synthase type II which involving in mikolat acid biosynthesis, component of cell wall of *M. tuberculosis*. Olugbuyiro et al. (2009) reported triterpenoid compounds derived from methanol extract of *Spondiasmombin* L. bark (Anacardiaceous family) has anti tuberculosis activity against *M. tuberculosis* H37Rv (sensitive strain) at extract concentration of 64 µg/mL.[14-17]

CONCLUSION

Both chloroform and Ethanol extract of Forest kedondong stembark were active as anti tuberculosis against MDR strain of *M. tuberculosis* with 100% inhibition at concentration of 100 mg/mL. Terpenoids and flavonoids might be contributed to the activity of these extracts. However, further research is suggested to identify anti tuberculosis constituents from these extracts.

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