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Antimicrobial activity of *Parkiaspeciosa*Hasskagainst Bacteria and Fungi using different Solvents Extractions

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Abstract:
Background: ParkiaspeciosaHassk is widely known as stink bean and one of the edible plants that have not been cultivated as medicinal plants yet
Materials and Methods: Samples were collected randomly from the local market in Kemaman, Terengganu. The pods were separated from the seed and cleaned thoroughly using tap water.
Extraction process by maceration using three solvents, n-hexane, ethyl acetate, and ethanol 95%. Qualitative phytochemical analysis of the extract was determined. Antimicrobial activity using Kirby-Bauer (KB) method.
Results: Maceration of ethanol 95% had the highest percent yield of 17.57% among all extracts.The phytochemical analysis resulted in the three extracts containing flavonoids. The three extracts with different solvents produced antimicrobial activity.
Conclusion: The results of the phytochemical analysis of the extract of ParkiaspeciosaHassk pods resulted in the content of flavonoid compounds, saponins, and tannins, which also have potential as antimicrobials. 95% ethanol extract has the highest potential as an antimicrobial.
Key Word: Antimicrobial, Parkia speciose Hassk, Kirby-Bauer Disc Susceptibility Test

I. Introduction

Natural products such as ParkiaspeciosaHassk. have too many benefits for humans. The plant is leguminous from the family of Fabacae, also placed into Leguminosae and Mimosaceae (Miyazawa and Osman. 2001). Based on various studies, ParkiaspeciosaHassk contains many properties, such as antioxidant and antimicrobial properties. The plant has many uses as the locals use them for many purposes, especially as an orthomolecular agent and for treating illnesses. The locals from Indonesia often use the waste material of this plant as an anti-inflammatory agent for mosquito bites (Hasim et al., 2015). The plant has an antimicrobial effect, especially on Gram-positive pathogenic bacteria such as Staphylococcus aureus, Bacillus cereus, and Listeria monocytogenes (Wonghirundecha et al., 2014).

The antimicrobial activity of this plant also is influenced by the types of solvent used to extract it because there is a study that stated that ethyl acetate gave the highest inhibition zone when compared to other extraction that has extract from n-hexane and ethanol at 70% (Hasim et al., 2015). There are many kinds of literature focusing on antioxidant activity but not on the antimicrobial activity

To use all parts of this plant, the pod would be a great source to investigate deeper the antimicrobial activity extracted using different types of solvent. This study aimed the efficacy of the pod of ParkiaspeciosaHassk against bacteria and fungi by using difference solvents.

II. Material And Methods

Sample collection and preparation:

Samples were collected randomly from the local market in Kemaman, Terengganu. The pods separated from the seed and they were cleaned thoroughly using tap water (Wonghirundecha et al., 2014). They were shade dried for 5 days. After drying, the pods were ground into fine powder by using a grinder (Gan &Latiff, 2011). Three different solvents, n-hexane, ethyl acetate, and ethanol 95% were used for extraction, for antimicrobial efficacy testing, microorganisms used in our study were Gram-positive bacteria (*Staphylococcus epidermidis*, *Bacillus subtilis*, and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumo*nia, and *Pseudomonas aeruginosa*) and the fungi (*Candida albicans*).

Solvent extraction:

Three solvents were used for the extraction process: n-hexane, ethyl acetate, and ethanol 95%. The method of extraction is maceration, the dried powder of ParkiaspeciosaHassk pod was mixed with a 1:5 solvent ratio for 24 hours with frequent agitation. After the extraction process, the solution was concentrated by evaporating solvent using a rotary evaporator (Rotavapor® R-300) at 60 °C with a final moisture content of less than 10% (Wonghirundecha et al., 2014).

Phytochemical screening

Qualitative phytochemical analysis of the extract was determined by the methods of screening tests of tannins, flavonoids, alkaloids, and saponin as described by Harborne (1987).

Antimicrobial activity

The extracted sample was tested for their antibacterial activity using Kirby-Bauer (KB) method against *Staphylococcus epidermidis* ATCC 51625, *Bacillus subtilis*, *Staphylococcus aureus* ATCC 33591, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 1706, *Pseudomonas aeruginosa* ATCC 15442, and *Candida albicans* ATCC 10231. In this test, bacteria culture solutions contain a turbidity standard of 0.5 x 106 CFU/ml prepared in normal saline and spread on sterile Mueller Hinton agar plates using the spread plate technique. Then discs containing different concentrations of extracts (50, 100, and 200 mg/mL) were placed on an agar plate where the selected bacteria were grown. A sterile blank disc containing DMSO was used as a negative control and ciprofloxacin as a positive control for bacteria and fungal DMSO was used as a negative control and Nystatin as a positive control. The plates were then incubated at 37°C for 24 hours. After incubation, the zone of inhibition was recorded. The experiment was repeated three times.

III. Result and Discussion

Yield from solvent extraction of ParkiaspeciosaHassk pod showed a high amount of ethanol 95%. The ethanol 95% yield was 17.57%, for ethyl acetate 5.26%, and for n-hexane 2.26%.

The qualitative phytochemical screening method detected the presence of a particular phytochemical compound in ParkiaspeciosaHassk pods extract. N-hexane extract only contained flavonoids. Ethyl acetate extract contained saponins, flavonoids, and tannin. Whereas ethanol 95% extract contained tannin, flavonoid, and saponins. These compounds were tested specifically because these compounds play a major role in acting as antibacterial agents(Tabel no 1)

Table no.1. Phytochemical screening of *Parkiaspeciosa*Hassk p

Phytochemical Test	**Solvent**		
	n-hexane	Ethyl acetate	Ethanol 95%
Alkaloid	-	-	-
Flavonoid	+	+	+
Saponin	-	+	+
Tannin	-	+	+

According to Hasim et al. (2015), flavonoids can destroy the bacteria as they could cause the membrane to leak out cell material, due to the action of flavonoids in causing the interference in bacteria cell membrane permeability. Saponins' role as an antibacterial agent in this plant might be because it can cause cell wall malfunctioning. The cell membrane activity will be limited, and this will cause the permeability of the membrane to destroyed, which will lead to the malfunctioned cell wall. Tannins can exhibit inhibition activity on the cell membrane, thus disrupting the normal functioning of essential enzymes and genetic material. Inhibition of the bacterial cell wall by tannins will be carry out because it will bind to the cell wall. The antifungal activity of flavonoids may influenceby the inhibitory action of fatty acid synthase during the gene pathway. Antifungal activity of flavonoids may be influence by the inhibitory action of fatty acid synthase during the pathway of the gene. On the other hand, saponins will cause the breaking down of membrane integrity by the pore formation on the fungal membrane because of the complexation that happens between saponins and sterol (Mert-türk, 2006). Tannins are widely known for their ability to bind to different membrane structures, and their antifungal activity, tannins have the affinity of binding to ergosterol, thus reducing the amount of ergosterol in fungal cells (Carvalho et al., 2018).

The antimicrobial activities of three different solvent were screened for their antimicrobial spectrum. The test bacteria used for screening were six bacterial cultures and one fungal. Table 2 summarizes the average microbial growth inhibition of the n-hexane, ethyl acetate, and ethanol 95% extracts. From the results, ethyl acetate's extract and ethanol 95% extract showed an inhibition zone, and n-hexane had no inhibition except for two concentrations of the extract against Staphylococcus aureus. This might be due to the sensitivity of the

extract depending on the level of the concentration when it is used against certain microorganisms. Between ethyl acetate's extract and ethanol 95%'s extract, it can be seen that ethanol 95%'s extract gave a higher inhibition zone compared to ethyl acetate's extract. This is due to theconcentration of the antimicrobial compounds extracted from ethanol 95% is higher than the extract from ethyl acetate. This could be explained by the influence of polarity of ethanol 95% being higher than ethyl acetate so this will affect the concentration of certain compounds obtained such as antimicrobial compounds, and ethanol could easily make its way towards the compounds as it can break the cell wall of cells (Calvo et al., 2007).

Table 2. Zone of Inhibition from Different Concentrations for Each Extracts

Microbes		Concentration	200 mg/mL	100 mg/mL	50 mg/mL	Ciprofloxacin/ Nystatin	DMSO
Gram + bacteria	*S. epidermidis*	N-hexane	5 mm	8 mm	8 mm	39 mm	-
		Ethyl acetate	19 mm	16 mm	11 mm	38 mm	-
		Ethanol 95%	20 mm	17 mm	14 mm	36 mm	-
	St. aureus	N-hexane	-	7 mm	8 mm	28 mm	-
		Ethyl acetate	15 mm	13 mm	10 mm	26 mm	-
		Ethanol 95%	16 mm	14 mm	12 mm	25 mm	-
	B. subtilis	N-hexane	-	-	-	33 mm	-
		Ethyl acetate	11 mm	10 mm	8 mm	32 mm	-
		Ethanol 95%	12 mm	11 mm	9 mm	32 mm	-
Gram - bacteria	*E. coli*	N-hexane	-	-	-	37 mm	-
		Ethyl acetate	-	-	-	36 mm	-
		Ethanol 95%	-	-	-	34 mm	-
	K. pneumonia	N-hexane	-	-	-	35 mm	-
		Ethyl acetate	12 mm	10 mm	9 mm	37 mm	-
		Ethanol 95%	12 mm	10 mm	8 mm	37 mm	-
	P. aeruginosa	N-hexane	-	-	-	45 mm	-
		Ethyl acetate	14 mm	11 mm	9 mm	45 mm	-
		Ethanol 95%	13 mm	11 mm	9 mm	46 mm	-
Fungal	*C. albicans*	N-hexane	-	-	-	18 mm	-
		Ethyl acetate	-	-	-	18 mm	-
		Ethanol 95%	-	-	-	18 mm	-

IV. Conclusion

The results of the phytochemical analysis of the extract of ParkiaspeciosaHassk pods resulted in the content of flavonoid compounds, saponins, and tannins, which also have potential as antimicrobials. 95% ethanol extract has the highest potential as an antimicrobial.

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