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REVIEW ARTICLE

Phytochemical analysis and antioxidant activity of different extracts of *Lawsonia inermis*: A mini review

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Abstract:

Lawsonia inermis is a member of the Lythraceae family and is known as "Hinai" in Malaysia and "Henna" in India. It has many traditional medicinal and pharmaceutical properties. The extraction method and solvent determine the extraction yield and antioxidant activity from plants. Thus, the purpose of this review is to provide an overview on how different extraction solvents affect the phytochemical and antioxidant properties of *L. inermis*. The phytochemical constituents recorded in qualitative studies include carbohydrate, protein, amino acid, sterols, saponins, alkaloids, tannins, flavonoid, anthraquinones, terpenoids, steroids, phenols, reducing sugar, glycoside, cardioglycosides, and oils. The solvent such as methanol, ethanol, acetone, chloroform, and aqueous are commonly used for plant extraction. Rather than the bark and fruit parts of *L. inermis*, the leaves and flowers were widely used and reported to reveal many phytochemical constituents. Antioxidant activity of *L. inermis* were measured using DPPH (1,1-Diphenyl-2-picrylhydrazyl), FRAP (ferric reducing antioxidant power), and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). Methanol and ethyl acetate extractions of *L. inermis* leaves and seeds, respectively, demonstrated the highest antioxidant activity in DPPH and FRAP assays. In conclusion, *L. inermis* extracts have high antioxidant properties and can be useful in both medicine and food industry. Future studies are needed to investigate the most suitable extraction solvents for each part of this valuable plant.

Keywords: Antioxidant, DPPH, FRAP, *Lawsonia inermis*, phytochemical

1. INTRODUCTION

Natural products remain an important source of synthetic medicine and traditional herbal medicine. According to Welz et al. (2018), the use of herbal medicine, as one element of complementary and alternative medicine is increasing worldwide. Traditional treatments, especially medicinal plants, continue to play a crucial role in addressing basic public health needs. In order to meet basic health needs in developing countries, traditional medicinal methods, in particular the use of medicinal plants, remain vital (Karunamoorthi et al., 2013). Herbal medicines in primary healthcare are in high demand both in developed and developing countries due to their large biological and medicinal activities, increased margins of safety and lower costs (Agarwal et al., 2014 as cited in Padma, 2005).

Lawsonia inermis, an Indian medicinal plant, is a perennial shrub native to India, North Africa, Asia, and Australia (Wagini et al., 2015). The genus Lawsonia bears one species, *L. inermis* (also known as Henna, Mhendi, Shudi, Madurang, Mendi, Manghati, Madayantika and Goranti) (Chaudhary et al., 2010; Wagini et al., 2015) till now, having different synonyms as alba and spinosa belonging to family Lythraceae. The scientific classification of *L. inermis* from kingdom until species is stated in Table 1.

Table 1. Scientific classification of *L. inermis*

Classification	Scientific name
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Lythraceae
Genus	*Lawsonia*
Species	*inermis*

Based on Figure 1, *L. inermis* is a tall shrub or small tree standing 1.8 to 7.6 m tall (6-25ft). The bark is greyish brown, the leaves are dull green and grow opposite each other's stem. The flowers are tiny, about 1 cm long, numerous, fragrant, white, or pink, with four crumbly petals. The fruit is a small round capsule coloured brown. The fruit contains many-seeded with 32-49 seeds per fruit and opens irregularly into four splits at maturity. Seeds are about 3 mm across, numerous, smooth, pyramidal, hard, and thick seed coat with brownish coloration (Triveni et al., 2016). In other studies carried out by Yadav et al. (2013), they believed that *L. inermis* was unarmed when young, but multiple branches of older trees were spine-tipped. Young branches are green in color and quadrangular, which turn red with age. They also stated that the leaves were indicated to be sub-sessile, around 1.5 to 5 cm long, 0.5 to 2 cm wide, greenish brown to dull green, elliptical to widely lanceolate with total margin, short and glabrous petioles and acute or obtuse apex with a tapering base.

Figure 1. *L. inermis* retrieved from Triveni et al. (2016).

L. inermis is now widely grown in the tropics as an ornamental and dye plant (Rani, 2018). According to Hasan et al. (2015), apart from Asian countries using *L. inermis* as a colouring agent in cosmetic products or textile process, the plant also owned wide range of pharmacological activities, safety, and availability. It has been noted that different parts of the *L. inermis* plant are a rich source of various bioactive principles and have been used in traditional medicine (Dasgupta et al., 2002).

It has been established that the hydroalcoholic extract of *L. inermis* possesses antioxidant activities (Ojewunmi et al., 2014). *L. inermis* has been shown to be rich in phenolic antioxidants such as lawsone, flavonoids, tannins and coumarins (Florence et al., 2015; Sadig et al., 2020). The plant has been reported to have antimalarial, antioxidant and antimicrobial properties (El Babili et al., 2013; Ponugoti, 2018 as cited in Afolayan et al., 2016). In addition, inventory of antidiabetic plants in Lagos State, Nigeria revealed that two percent of traditional practitioners have used *L. inermis* leaves in the management of diabetes (Gbolade, 2009).

Many extraction techniques have been utilized to recover antioxidants from plants which include Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction (Do et al., 2014). However, extraction yield and antioxidant activity are not only affected by the extraction method but also the solvents used for extraction. Previous studies have used methanol, n-hexane, chloroform, ethanol, acetone, and water as the solvents for extracting bioactive compounds from *L. inermis* for phytochemical and antioxidant analysis and the results varied between each solvent (Nounah et al., 2017; Sadig et al., 2020; Meghmala et al., 2019). Thus, this study aims to review the phytochemical and antioxidant properties of plant extract of *L. inermis* in different types of extraction solvents.

2. DISCUSSION

Solvents such as methanol, n-hexane, chloroform, ethanol, acetone, and water are commonly used in plant extraction. The extraction solvent is also the most common method used to remove phenolic antioxidants, and both extraction yield and extraction activity rely heavily on the solvent. The antioxidant capacity of phenolic compounds is greatly influenced by the solvent polarity used in extraction. Therefore, choosing extraction solvents is essential for complex plant samples. The extraction solvent system is usually selected for the extraction purpose, the polarity of the components involved, the polarity of undesirable components, the overall cost, safety, and environmental issues (Tan et al., 2013 as cited in Wang et al., 2008).

2.1. Phytochemical Analysis of *L. inermis* in Different Extraction Solvents

2.1.1. Methanol extract

Methanol is an alcohol (-OH) group dominant solvent. It is usually used in medicinal plants for extraction to search for bioactive because of its protic polar solvent. Most of the active compounds of plants are soluble in methanol (Felhi et al., 2017). In a study conducted by Sharma and Goel (2018), they use *L. inermis* leaves that were collected in India. It had been reported that the methanol extract of *L. inermis* leaves shown a presence of alkaloids, cardioglycosides, carbohydrate, steroids, phenol and quinones but an absence of flavonoids.

However, Kimbonguila et al. (2019), found that there was no cardioglycosides present but show the presence of flavonoids in the methanolic extract of *L. inermis* leaf and also revealed the presence of different phytochemical constituents in this plant. These differences may occur due to the different methods of determination of flavonoids used. Sharma and Goel (2018) used alkaline test to detect flavonoids and Legal's test to detect cardioglycosides. On the other hand, Kimbonguila et al. (2019), did not state the method used and were claimed to be assessed with standard methods to detect flavonoids and cardioglycosides. In addition, Nesa et al. (2014) studies show the presence of flavonoids, glycosides,

phytosterol, steroids, and tannins in *L. inermis* barks. Based on this finding, it can be concluded that both barks and leaves of *L. inermis* contain flavonoids, glycosides, steroids, and tannins.

2.1.2. Ethanol extract

According to Dai & Mumper (2010), ethanol is considered an excellent polyphenol extraction solvent and is suitable to be consumed by humans. Besides, it is found easier to penetrate the cellular membrane to extract or remove the intracellular ingredients from the plant material. In a study conducted by Jeyaseelan et al. (2012), the flower, leaf, and fruit parts from *L. inermis* show the presence of flavonoids, saponins and tannins in both flower and fruit. However, only flavonoids and tannins were presence in the leaf. This result contrasted with the finding of Rao et al. (2016) as flavonoids, saponins and tannins were presence in leaf. The different regions of plant collection may contribute to this factor as *L. inermis* leaves from both researchers were collected at Sri Lanka and Osmania, respectively. According to Yusuf (2016), the *L. inermis* leaves from India revealed the absence of tannins, which opposites from the finding of both researchers. The presence of flavonoids, saponins and tannins in flower by Rao et al. (2016) are consistent with the finding of Fathima (2018). This finding shows that ethanol can yield a wider phytochemical constituent in a plant, making it the best extraction solvent for *L. inermis*.

2.1.3. Acetone extract

Acetone, a polar molecule, is organic, non-toxic, and extremely flexible, making it an essential solvent for washing, sterilization, extraction, and chemical research activities. In a study conducted by Chowdhury et al. (2014), *L. inermis* leaf extracted with acetone revealed the presence of cardioglycosides, terpenoids, carbohydrates, phenols, quinones, and tannins. In their results, there was no presence of terpenoids. This finding is also supported by Gull et al. (2013), where the leaf of *L. inermis* also shows the presence of cardioglycosides, terpenoids, carbohydrates, phenols, quinones, and tannins. In addition, other phytochemicals such as flavonoids, phlobatanins, steroid, and volatile oil were found in the acetonic leaf extract of *L. inermis* (Rao et al., 2016; Sharma et al., 2009). Based on this finding, it can be concluded that acetone was commonly used in leaves extraction of *L. inermis* and shows the presence of tannins, flavonoids, terpenoids, saponins, cardioglycosides, glycosides, phlobatanins, steroid, phenolic, proteins, quinones, steroids and volatile oil.

2.1.4. Chloroform extract

Chloroform is a colorless, volatile, liquid derivative of trichloromethane with an ether-like odor. Besides, chloroform is known as a non-polar solvent. According to Leela & Singh (2020), the *L. inermis* leaf collected from Nagercoil, India with chloroform extraction revealed the presence of alkaloid, glycoside, cardiac glycoside, terpenoids, diterpenes, lipids, steroid, phytosterol, resin, fixed oil and fats, carboxylic acid, carbohydrate, and starch. In contrast to Florence et al. (2015), the researchers did not specify the location where the leave was collected and claim to collect the samples at different localities in India. Their study on chloroform extraction of *L. inermis* leaves revealed the presence of carbohydrates, coumarins, phenols, phytosterols, proteins, quinones, sterols and terpenoids but the absence of alkaloids and glycosides in the leaves of *L. inermis*. Though the leaf was taken from the same country in India, the location it was collected is different, resulting in varied phytochemical screening. In another study by Chowdhury et al. (2014), the leaves collected in Bangladesh show the presence of cardioglycosides, tannins, carbohydrates, phenol and quinones but an absence of terpenoids.

2.1.5. Aqueous extract

In a previous study conducted by Leela & Singh (2020), the presence of alkaloids, glycosides, cardiac glycosides, terpenoids, diterpenes, lipids, steroid, phytosterol, quinones, carboxylic acids, carbohydrates, phenols, tannins, flavonoids, coumarins, saponin, phlobotannins, proteins, and amino acids was reported in the leaf part of *L. inermis*. In contrast to the study of Meghmala et al. (2019), they revealed that there were only presence of flavonoids, phenols, and steroids. Meanwhile, in another study by Fathima (2018), the flower of *L. inermis* were reported the presence of alkaloids, carbohydrates, flavonoids, tannins, proteins, amino acids, and sterols in the. Based on these findings, flower and leaf part of this plant produced alkaloids, carbohydrates, flavonoids, tannins, proteins, and amino acids. Table 2 summarises the phytochemical screening of *L. inermis* in the different extract solvents.

2.2. Antioxidant Activity of *L. inermis* in Different Extraction Solvents

According to Chaves et al. (2020), there are several methods that can be used to quantify antioxidant activity which can be categorised depending on the mechanism of action used by the compounds to stop chain-breaking reactions. They can be classified into two main categories which are single electron transfer (SET) and hydrogen-atom transfer (HAT. The most used SET techniques include the 2,2-di-phenyl-1-picrylhydrazyl (DPPH radical scavenging capacity assay), ferric reducing (FRAP), Trolox equivalent antioxidant capacity (TEAC or ABTS), copper reduction (CUPRAC), and reducing power assay (RP). The crocin bleaching assay, the total oxyradical scavenging capacity (TOSC), the oxygen radical absorbance capacity (ORAC), and the total peroxyl radical-trapping antioxidant parameter (TRAP) are examples of hydrogen atom transfer reaction assays. The antioxidant activities of different parts of *L. nermis* in the various extraction solvents are summarised in Table 3.

Table 2. Phytochemical screening of *L. inermis* in different extract solvents

Extraction Solvent	Part of Plant	Phytochemicals	References
Methanol	Bark	Flavonoids, glycosides, phytosterol, steroids, and tannins	Nesa et al. (2014)
	Leaves	Alkaloids, cardioglycocides, carbohydrates, steroids, phenol and quinones	Sharma and Goel (2018)
	Leaves	Alkaloids, terpenoids, flavonoids, tannins, phenolic, saponins, fixed oil and fats	Kimbonguila et al. (2019)
Ethanol	Flower	Flavonoids, saponins, and tannins	Jeyaseelan et al. (2012)
	Flower	Flavonoids, saponins, tannins, alkaloids, cardiac glycosides, glycosides, proteins, amino acids, fixed oil, fats, steroids and terpenoids	Fathima (2018)
	Leaves	Flavonoids and tannins	Jeyaseelan et al. (2012)
	Leaves	Flavonoids, saponins, alkaloids, steroids, terpenoids, tannins, cardiac glycosides, glycosides, reducing sugars, phlobatanins, steroids, phenolic, amino acids, proteins, quinones	Rao et al. (2016)
	Leaves	Flavonoids, alkaloids, saponins, terpenoids, and steroids	Yusuf (2016)
	Fruit	Flavonoids, saponins, and tannins	Jeyaseelan et al. (2012)
Acetone	Leaves	Flavonoids, saponins, steroids, volatile oils, tannins, carbohydrates.	Sharma et al. (2009)
	Leaves	Cardioglycosides, terpenoids, carbohydrates, phenols, quinones, and tannins	Gull et al. (2013)
	Leaves	Cardioglycosides, terpenoids, carbohydrates, phenols, quinones, and tannins	Chowdhury et al. (2014)
	Leaves	Tannins, flavonoids, terpenoids, saponins, cardioglycosides, glycosides, phlobatanins, steroid, phenolic, proteins, and quinones	Rao et al. (2016)
Chloroform	Leaves	Cardioglycosides, tannins, carbohydrates, phenol and quinones but an absence of terpenoids	Chowdhury et al. (2014)
	Leaves	Alkaloid, glycoside, cardiac glycoside, terpenoids, diterpenes, lipids, steroid, phytosterol, resin, fixed oil and fats, carboxylic acid, carbohydrate, and starch	Leela & Singh (2020)
	Leaves	Carbohydrates, coumarins, phenols, phytosterols, proteins, quinones, sterols and terpenoids	Florence et al. (2015)
Aqueous	Flower	Alkaloids, carbohydrates, flavonoids, tannins, proteins, amino acids, and sterols	Fathima (2018)
	Leaves	Flavonoids, phenols, and steroids	Meghmala et al. (2019)
	Leaves	Alkaloids, glycosides, cardiac glycosides, terpenoids, diterpenes, lipids, steroid, phytosterol, quinones, carboxylic acids, carbohydrates, phenols, tannins, flavonoids, coumarins, saponin, phlobotannins, proteins, and amino acids	Leela & Singh (2020)

Table 3. Antioxidant activity of *L. inermis* in different extraction solvents

Antioxidant Activity	Part of Plant	Extraction solvent	Results	References
DPPH	Leaves	Hexane	EC_{50} > 200 µg/mL	Hsouna et al. (2011)
		Chloroform	EC_{50} > 200 µg/mL	
		Ethyl acetate	EC_{50} = 4.8 ± 0.2 µg/mL	
		Water	EC_{50} = 7.6 ± 2.1 µg/mL	
	Leaves	Methanol	70.16 %	Radha et al. (2017)
	Leaves	Methanol	71.7 ± 0.02 %	Meghmala et al. (2019)
	Seed	Hexane	IC_{50} > 100 µg/mL	Chaudhary & Kalia (2014)
		Chloroform	IC_{50} > 100 µg/mL	
	Seed	Hexane and chloroform	IC_{50} > 100 µg/mL	Nounah et al. (2017)
	Seed	Hexane	IC_{50} > 100 mg/L	Chaibi et al. (2017)
		Chloroform	IC_{50} > 100 mg/L	
		Methanol	IC_{50} = 4.6 ± 0.2 mg/L	
FRAP	Leaves	Ethyl acetate	IC_{50} = 486 µg/mL	Kumar et al. (2014)
		Butanol	IC_{50} = 504.76 µg/mL	
		Chloroform	IC_{50} = 782.49 µg/mL	
		Methanol	IC_{50} = 900.83 µg/mL	
	Leaves	Methanol	1101 ± 0.02 µg/Trolox equivalent	Meghmala et al. (2019)
	Whole	Aqueous	IC_{50} = 313.93 ± 0.39 µg/mL	Guha et al. (2011)
		Methanol	IC_{50} = 430.8 ± 0.35 µg/mL	
		Chloroform	IC_{50} = 1815.67 ± 0.11µg/mL	
ABTS	Leaves	Ethanol	IC_{50} = 6.9 ± 0.1 mg/L	El Babili et al. (2013)
		Petroleum ether	IC_{50} = 738.7 ± 9.6 mg/L	
		Ethyl acetate	IC_{50} = 8.6 ± 0.2 mg/L	
	Leaves	Ethanol	IC_{50} = 1.29 µg/mL	Kumar et al. (2014)
		Methanol	IC_{50} = 1.43 µg/mL	
		Butanol	IC_{50} = 2.90 µg/mL	
		Chloroform	IC_{50} = 27.24 µg/mL	
		Hexane	IC_{50} = 174.3 µg/mL	
		Aqueous	IC_{50} = 219.47 µg/mL	
	Seed	Methanol	IC_{50} = 3 ± 1.3 mg/L	Chaibi et al. (2017)
		Chloroform	IC_{50} > 100 mg/L	
		Hexane	IC_{50} > 100 mg/L	
	Seed	Hexane	IC_{50} = 1283.8 ± 7.4 µg/mL	Nounah et al. (2017)

2.2.1. DPPH scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay is a fast, easy, and economical colorimetric test to measure antioxidants' ability to reduce the DPPH radical. DPPH is widely used to measure the ability of compounds to serve as scavengers of free radicals and to determine foods' antioxidant activity. In antioxidant activity studies of plant extracts, the DPPH approach has been widely used (Chaves et al., 2020). In addition, this activity was also calculated by evaluating the IC_{50} or EC_{50} values corresponding to the concentration of sample needed to scavenge 50 percent of the sum of the initial DPPH radicals in the mixture of the reaction. The higher the IC_{50} or EC_{50} value, the lower the antioxidant activity of the sample tested (Hsouna et al., 2011; Dhaouadi et al., 2015).

According to Meghmala et al. (2019), methanolic extract of *L. inermis* leaf showed to have antioxidant properties when tested with DPPH scavenging activity which resulted in 71.7 ± 0.02 % DPPH scavenged. This finding is also supported by Radha et al. (2017), where the antioxidant scavenging activity for the leaf part in methanolic extract of *L. inermis* resulted in 70.16 %. In their results, 50 mL of extract sample can produce scavenging activity of 44.20 %. This shows that the methanolic extract of *L. inermis* leaves contain higher antioxidant activity even in low concentration which, according to the researcher, may occur due to higher total phenolic content. On the other hand, a study conducted by Hsouna et al. (2011), values of EC_{50} in the DPPH free radical-scavenging of *L. inermis* leaf extracted with hexane, chloroform, ethyl acetate, and water shows $EC_{50} > 200$ µg/mL, $EC_{50} > 200$ µg/mL, $EC_{50} = 4.8 \pm 0.2$ µg/mL, and $EC_{50} = 7.6 \pm 2.1$ µg/mL, respectively. This finding suggested that hexane and chloroform are not the best extraction solvent for observing the antioxidant activity of *L. inermis* because both solvents did not show any antioxidant activities.

Another study conducted by Chaibi et al. (2017), values of IC_{50} in the DPPH free radical scavenging of *L. inermis* seed extracted with hexane, chloroform, and methanol are $IC_{50} >$ 100 mg/L, $IC_{50} > 100$ mg/L, and $IC_{50} = 4.6 \pm 0.2$ mg/L, respectively. Studies conducted by Chaudhary & Kalia (2014) and Nounah et al. (2017) show that antioxidant activity of chloroform and hexane for seed extraction of *L. inermis* display the IC_{50} value more than 100 µg/mL. Based on this finding, it can be concluded that methanol is a better solvent than other solvents for a more consistent extraction of antioxidants from *L. inermis* leave and seeds.

2.2.2. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay is based on the potential of samples to reduce Fe^{3+} to Fe^{2+} (Spiegel et al., 2020). According to Meghmala et al. (2019), the absorbance of Soxhlet methanol of leaf extract at 593nm was shown a higher antioxidant potential than aqueous and Soxhlet water extracts. For *L. inermis* leaf in Soxhlet methanol extraction, 1101 ± 0.02 µg per trolox equivalent were reported. However, aqueous extract of *L. inermis* leaf, was shown to have the lowest reducing power with 400.66 ± 0.04 µg per trolox equivalent. This finding is also supported by Kumar et al. (2014), where methanol extraction of *L. inermis* leaf was also reported to have higher reducing power than water extraction. However, according to Kumar et al. (2014), ethyl acetate (IC_{50} = 486 µg/mL) was found to have the greatest reduction power followed by butanol (IC_{50} = 504.76 µg/mL), chloroform (IC_{50} = 782.49 µg/mL), and methanol (IC_{50} = 900.83 µg/mL). Hexane and aqueous were found to be least active when compared to the rutin equivalent and both solvents did not attain 50 % inhibition even at the maximum concentration measured. In contrast with the study of Guha et al. (2011), the use of a whole plant of *L. inermis* was extracted using a Soxhlet apparatus. In their study, *L. inermis* plant in aqueous extraction shown the highest activity (IC_{50} = 313.93 ± 0.39 µg/mL) compared to methanol (IC_{50} = 430.8 ± 0.35 µg/mL) and chloroform with the least antioxidant efficiency (IC_{50} = 1815.67 ± 0.11µg/mL). These differences may be due to different phenolic compounds distribution in different parts of the plant, directly contributing to antioxidant action.

In another study conducted by Chaudhary & Kalia (2014), the results showed for 50 µg/ml of *L. inermis* seed sample that out of different extract, the absorbance of ethyl acetate (0.165) showed the highest reducing power followed by ethanol (0.144), aqueous (0.094) and chloroform (0.042). This finding is supported by Philip et al. (2011), the absorbance of ethanol extract of *L. inermis* seed was shown higher reducing power than aqueous extract. In the same study, petroleum ether extract of *L. inermis* seed revealed the lowest reducing power compared to ethanol and aqueous extract. It is important to note that both Chaudhary & Kalia (2014) and Philip et al. (2011) used ascorbic acid as reference standards. The reducing capacity of a compound can serve as the antioxidant activity, with the reducing power of extracts are directly proportional with extract concentration. Increased absorption of the reaction mix means an increase in the sample reduction power. Based on this finding, it can be concluded that in FRAP assay, ethyl acetate is the best extraction solvent for leaf and seed of *L. inermis*. The successful reduction of the extract of ethyl acetate demonstrates its efficacy in preventing oxidation. Naturally occurring reductants are active in the processes of oxidative protection, and reduction ability may serve as an essential indicator of their potential antioxidants.

2.2.3. ABTS scavenging activity

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay is also commonly used to assess the antioxidant potential of natural products. According to El Babili et al. (2013), the study on extraction yields and antioxidant activity of *L. inermis* leaf was investigated *in vitro*. The ethanol extract of *L. inermis* leaf showed an antioxidant activity IC_{50} of 6.9 ± 0.1 mg/L. The least antioxidant extract with IC_{50} was petroleum ether extract with 738.7 ± 9.6 mg/L. The ethyl acetate extract exhibited an IC_{50} of 8.6 ± 0.2 mg/L. This

finding showed that ethanol extracts yield the highest antioxidant for the leaf part of *L. inermis* in ABTS assay compared to other solvents used in the study. This result was in line with Kumar et al. (2014) where all extracts and fractions of *L. inermis* leaf demonstrated the ability to scavenge ABTS radicals and the highest antioxidant activity was shown in the ethanolic extracts.Their activity based on IC_{50} values was in the order: ethanol (IC_{50} = 1.29 µg/mL) > methanol (IC_{50} = 1.43 µg/mL) > butanol (IC_{50} = 2.90 µg/mL) > chloroform (IC_{50} = 27.24 µg/mL) > hexane (IC_{50} = 174.3 µg/mL) > aqueous (IC_{50} = 219.47 µg/mL). Based on this finding, in ABTS assay, ethanol solvent could be the best for measuring antioxidant activity for the leaf part of *L. inermis*.

In another research by Chaibi et al. (2017), the seed extracts of *L. inermis* were tested for antioxidant activity by individual seed. The antioxidant activity of seed extracts according to the ABTS assay was more significant by methanol (IC_{50} = 3 ± 1.3 mg/L). Extracts of chloroform and hexane display no antioxidant activity (IC_{50} > 100 mg/L), which corresponded to the result of Nounah et al. (2017), where the anti-radical activity of *L. inermis* seed oil extracted by hexane exhibited a very weak antioxidant activity (IC_{50} = 1283.8 ± 7.4 µg/mL). Even though it is a mixture of several compounds, the IC_{50} is comparable to the vitamin C of ABTS (IC_{50} = 3 ± 1.6 mg/L) (Chaibi et al., 2017). They proposed that the high levels of total phenolics in polar extracts were primarily responsible for the antioxidant activity of *L. inermis*. Based on this finding, it can be concluded that different parts of *L. inermis* may require a different solvent to observe better antioxidant properties in ABTS assay. This variation might be due to the different polarities of the different compounds in the plant extracts.

3. CONCLUSION

In conclusion, the extraction solvents play an important role in the extraction of bioactive groups from *L. inermis*. Solvent attracts various plant compounds based on several variables, such as polarity, boiling temperature, heat, oxygen and light reactivity, viscosity, and stability. Methanol, ethanol, and ethyl acetate are commonly suggested as acceptable extraction solvent for antioxidant assays such as DPPH, FRAP and ABTS. Many phytochemical constituents of antioxidant potential have been identified for all parts of the *L. inermis*, including leaves, barks, flowers, and seeds. Therefore, *L. inermis* has a significant advantage in treating diseases since it has many phytochemical components and antioxidant abilities. Future studies are required to identify the most suitable extraction solvents for each part of this valuable plant.

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