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Original Article

PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY ON SELECTED INDONESIAN WEEDS EXTRACTS: A NOVEL INSIGHT TO ANTI-SHIGELLOSIS

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ABSTRACT

Objective: Elephant grass (*Pennisetum purpureum* S.), weed grass (*Imperata cylindrica* L.), pearl grass (*Hedyotis corymbosa* L.) and nut grass (*Cyperus rotundus* L.) are selected weeds found in Indonesia which have been used as ruminants feeding with a complete diet component and evidently reported that bioactive contents of weeds provide more protection to microbial attack than that of crops. This has led to an increase interest in the investigation of weed extracts as anti-shigellosis agents for humans and animals, but there is still no data regarding on phytochemical and pharmacological of our selected weeds as an anti-shigellosis. Therefore, the objectives of this study was to analyze phytochemical and anti-shigellosis properties of those selected weeds towards sensitive (S) and resistant *S. dysentriae* (R) strains of ampicillin, chloramphenicol, and cotrimoxazoles.

Methods: Phytochemical screening was done using the standard method and further analyzed by thin-layer chromatography (TLC). The anti-shigellosis activity was evaluated using the agar diffusion method; meanwhile, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) value was determined using the microdilution method.

Results: In general, weeds contain flavonoids, steroid, and quinone compounds. The resulted anti-shigellosis showed that all weed extracts produced higher inhibition to sensitive than resistant strains. The MIC-MBC values of each weed on sensitive and resistant, respectively, were as follow: *P. purpureum* S (S=≥1.25%; R=≥2.5% w/v); *I. cylindrica* (S=≥5.0%; R=≥ 2.5-10.0%w/v); *H. corymbosa* (S=≥2.5%; R=≥2.5-10%w/v); and *C. rotundus* (S=≥2.5-5.0%; R=≥5.0-10%w/v). From these data, all of these weeds have the potential to complement antibiotics that are no longer effective in the treatment of shigella infections.

Conclusion: In summary, *P. purpureum* extract could be promoted as a novel supplement phytopharmaceutical for the treatment of bacillary dysentery.

Keywords: *Pennisetum purpureum* S., *Imperata cylindrica* L., *Hedyotis corymbosa* L., *Cyperus rotundus* L., Shigella dysentriae, Resistant

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INTRODUCTION

Shigellosis is a gastrointestinal disease form of bacterial diarrhea mix with fever caused by the Shigella species, notably occur in children [1, 2]. It can be direct spread from a person with poor sanitation, or transmitted from the ingestion of contaminated food. In 2018, WHO has been noticed this infection as priority pathogen due to the increasing of antibiotic resistance, no avalaible vaccine and high mortality burden approximately 13.2% of all diarrhoeal deaths worldwide [3, 4]. Without proper treatment, shigellosis can progress to a life-threatening systemic disease called hemolytic uremic syndrome, which is characterized by thrombocytopenia, hemolytic uremia, and kidney failure [5].

Treatment of shigellosis can be administered with antimicrobial agents such as tetracycline, ampicillin, cotrimoxazole, chloramphenicol, ciprofloxacin, pivmecillinam, ceftriaxone and azithromycin [6, 7]. In Indonesia, *S. dysenteriae* have been reported to be resistant against ampicillin (82%), cotrimoxazole (84%), and chloramphenicol (82%) [8]. In addition, this resistance also reported for other antibiotics, such as: ciprofloxacin, ceftriaxone, and azithromycin [9, 10]. Current WHO guidelines recommend to choose fluoroquinolones (first-line), β-lactams (second-line) and cephalosporins (second-line) which considered to have better effectiveness [1]. But unfortunately, they also found to be resistant to the current treatment in some countries [11, 12]. Therefore, an appropriate complement drug to face the era of increasing anti-shigellosis resistance is important to be found. This condition encourages scientists to investigate for new sources of anti-shigellosis agents from various sources such as herbal materials.

Several plant families are known to have antidysenteric activity, including the Poaceae family, such as species *Desmostachya bipinnata* L. and *Cyanodon dactylon* [13, 14]; Rubiaceae family such as the species *Nauclea latifolia* Sm. and *Paederia foetid* L. [15, 16]; and the family Cyperaceae, such as *Cyperus rotundus* Linn and *Cyperus tegetum* [17, 18]. Several types of grass belonging to the above family are known to be commonly used as the main feed for ruminants. According to a survey conducted by researchers on Indonesian farms in Lembang district, it shows that livestock in that location are very rarely exposed to infectious diseases such as dysentery. This probably suspected that the livestock's resistance comes from the grass they consumed. Elephant grass (*Pennisetum purpureum* S.), weed grass (*Imperata cylindrica* L.), pearl grass (*Hedyotis corymbosa* L.) and nut grass (*Cyperus rotundus* L.) are selected weeds found in Indonesia which have been used as ruminants feeding with a complete diet component and evidently reported that bioactive contents of weeds provide more protection to microbial attack than that of crops [19-21]. This has led to an increase the interest in the investigation of weed extracts as anti-shigellosis agents for humans and animals, but there is still no data regarding on phytochemical and pharmacological of our selected weeds as an anti-shigellosis. This study will offer a novel insight of new plants that were not only effective against S. *dysentriae* in general but also that were resistant to several anti-shigellosis antibiotics that had been used so far with a broad-spectrum. Therefore, in this study, we used three isolates of *S. dysenteriae* obtained from food and beverages were resistant to several antibiotics such as ampicillin, chloramphenicol, and cotrimoxazole which isolated from our previous work.

MATERIALS AND METHODS

Plant materials

The plant materials were consisted of Elephant grass (*Pennisetum purpureum* S.), weed grass (*Imperata cylindrica* L.), pearl grass (*Hedyotis corymbosa* L.) and nut grass (*Cyperus rotundus* L.), which was identified at the Department of Biology, Padjadjaran University with reference no. 66/HB/02. The weed used were fresh weed collected from the Manoko plantation, Lembang, West Java.

Bacterial strains and growth medium

S. dysenteriae (ATCC 13313) strain and three isolates of *S. dysenteriae* (1st generation) obtained from food and beverages which were resistant to several antibiotics such as ampicillin (isolate 1), chloramphenicol (isolate

2), and cotrimoxazole (isolate 3). The tested bacteria were maintained in Shigella-Salmonella (SS) (Pronadisa), Mueller-Hinton agar (MHA) (Merck) and Mueller-Hinton broth (MHB) (Oxoid).

Sample collection, processing and extraction

The fresh weed is then dried at a temperature below 30 °C to avoid decomposition of the thermolabile chemical components. Weed must be protected from direct sunlight because of the potential for chemical transformation caused by ultraviolet radiation. To prevent heat and humidity from building up, air circulation around the weed is essential. The weed is not piled up, and if necessary a fan is used to regulate airflow while drying the weed. After drying, the dried weed was chopped to improve the extraction efficiency by increasing the surface area. Chopping also reduces the amount of solvent used because dried weed can be loaded more densely. Each dried weed was weighed and then extracted by maceration method using 70% ethanol as solvent. After soaking with fresh solvent, the dried weed was kept for 24 h. After that the solvent was transferred through a filter and then a new fresh solvent was added, stirred and left overnight. This process was carried out for 24 h in three times. After replacing the solvent three times, the chemical components of the plant were almost completely used up. All the liquid extract obtained was then concentrated with a rotary evaporator at a temperature of 40 °-50 °C and continued with re-evaporation over a water bath at a temperature of 40 °C until the weight of the extract was constant. Each plant was given the same treatment. Then, the water content of the extract was determined using the distillation of toluene [22].

Phytochemical screening

Phytochemical screening was carried out to determine the group of compounds contained in each thick extract of the weed using a standard method, including alkaloids. flavonoids. tannins, saponins, polyphenols, quinones, monoterpenoids and sesquiterpenoids, triterpenoids and steroids [23].

Thin layer chromatography (TLC)

Thin Layer Chromatography (TLC) profile of weed ethanol extract was determined using a stationary phase in the form of a silica gel plate GF 254 and a mobile/developer phase in the combination of n-hexane: ethyl acetate (40: 60) and ethyl acetate: methanol (60: 40) solvents. On the starting line (1 cm from the edge) of a 10 x 2 cm silica gel plate, the ethanol extract was spotted using a capillary tube. The plate is left for some time until the solvent evaporates. The plate was placed in a chromatographic vessel, which has been previously saturated with the developer solution. The chromatographic process was stopped when the developer liquid reaches the finish line. The chromatogram pattern was observed in visible light, under UV lamps at 254 and 366 nm. Each observed spot was calculated as its Rf value [24].

Antibacterial activity test

The antibacterial activity of each weed extract was performed using the agar diffusion method. The weed extract stock solution was reconstituted using 10% DMSO, then diluted serially starting from 800 mg/ml. Bacterial suspension was prepared by inoculating 2 or 3 Ose of colonies from bacterial slant agar into a 5 ml of sterile physiological NaCl. The turbidity of bacterial suspension was adjusted to achieve a concentration of 1.5 $\times 10^8$CFU/ml (0.5 McFarland's standard). The bacterial suspension and 0.5 McFarland's standard were hold in front of light on a white background with contrasting black lines. If the bacterial suspension is too turbid, the suspension should be diluted with sterile physiological NaCl. Conversely, if the density of bacterial suspension was too light, then some Ose of bacterial colonies was taken into the suspension and compared to 0.5 McFarland's standard. A 20 µl of prepared bacterial suspension was poured into a sterile petri-disc containing 20 ml liquid MHA, then homogenized and allowed it to be solidified. The inoculated plates then perforated to make holes as the extract storage in certain concentration (200-800 mg/ml) in a volume of 50 µl. The test was done in triplicates. The plates then incubated at 37 °C for 24 h. The diameter of inhibition zones was measured [25].

MIC and MBC determination

MICs of the weed extracts against all tested *S. dysentriae* were determined by microdilution assay using 96-well microtiter plates. The wells were filled with 100 µl sterile MHB, then the extract in a volume of 100 µl was serially diluted in a two-fold dilution, starting from 100 to 1.5625 mg/ml with sterile MHB as the diluent. Subsequently, 100 µl of the last concentration was discharged, thus the tested medium per well was 100 µl. Each well then inoculated with 100 µl bacterial suspension ($1x10^4$cfu/ml), except the negative control well. The inoculated microdilution plate was then incubated at 37 °C for 24 h. The turbidity of each well was observed to determine the MIC value of the extract. Then 10 µl of MIC result sample was subcultured on to the surface of MHA and incubated at 37 °C for 24 h. This subculture method was performed to determine the MBC values of the extracts by observing the presence of bacterial colonies [26].

RESULTS

The characteristics of the extract are yellowish black, bitter, sweet smelling, and thick for elephant grass; yellowish black, bitter, sweet smelling, and thick for weeds grass; greenish-black, bitter, fragrant with tea, and thick to pearl grass; black-green, bitter, sweet-smelling, and thick for nut grass. Phytochemical screening was carried out to determine the class of secondary metabolites contained in the extracts, presented in table 1.

From optimization results, it was found that the mobile phases have good resolution, including: n-hexane: ethyl acetate (40: 60) and ethyl acetate: methanol (60: 40). Thus, both mobile phases were chosen in determining the chromatographic profile. The results were shown in fig. 1-2 and table 2-3.

Table 1: Yield of extract and phytochemical contents

Plant	% yield	Water content (%)	Phytochemical contents						
			Alkaloids	Flavonoids	Saponins	Tannins	Polyphenol	Steroid	Quinones
Pennisetum purpureum S.	14.82	25	-	+	+	+	+	+	+
Imperata cylindrica L.	11.04	20	-	+	-	-	-	+	+
Hedyotis corymbosa L.	12.42	15	-	+	-	+	-	+	+
Cyperus rotundus L.	7.80	15	-	+	-	-	-	+	+

Notes: (+) presence; (-) absence

Table 2: TLC results with n-hexane: ethyl acetate (40: 60) as the mobile phase

Spot No.	Rf	Visible light	UV light		Detection on extract of
			254 nm	366 nm	
1	0.06	-	-	Orange	HC
2	0.08	-	-	red	PP, IC, HC, CR
3	0.20	-	-	red	PP, IC, HC, CR
4	0.46	-	-	blue	HC
5	0.74	-	-	red	PP, IC, HC, CR
6	0.86	-	-	red	PP, IC, HC, CR

Notes: *Pennisetum purpureum* S. (PP); *Imperata cylindrica* L. (IC); *Hedyotis corymbosa* L. (HC); *Cyperus rotundus* L. (CR)

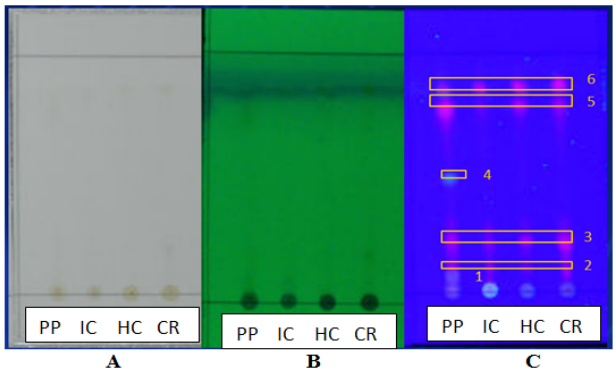

Fig. 1: TLC Profile of weeds ethanol extract with n-hexane: ethyl acetate (40: 60) as the mobile phase. Notes: *Pennisetum purpureum* S. (PP); *Imperata cylindrica* L. (IC); *Hedyotis corymbosa* L. (HC); *Cyperus rotundus* L. (CR); A. Visible light; B. 254 nm UV light; C. UV light 366 nm; 1-6= Rf

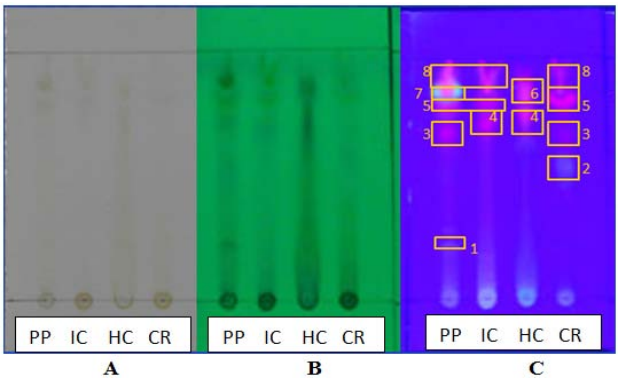

Fig. 2: TLC Profile of weeds ethanol extract with ethyl acetate: methanol (60: 40) as the mobile phase. Notes: *Pennisetum purpureum* S. (PP); *Imperata cylindrica* L. (IC); *Hedyotis corymbosa* L. (HC); *Cyperus rotundus* L. (CR); A. Visible light; B. 254 nm UV light; C. UV light 366 nm; 1-6= Rf

Table 3: TLC results with ethyl acetate: methanol (60: 40) as the mobile phase

Spot No.	Rf	Visible light	UV light		Detection on an extract of
			254 nm	366 nm	
1	0.20	-	brown	Light blue	HC
2	0.44	-	brown	blue	PP
3	0.72	yellow-green	brown	red	HC, PP
4	0.74	yellow-green	brown	red-orange	CR, IC
5	0.82	yellow-green	brown	red	HC, CR, PP
6	0.86	yellow-green	brown	red-orange	IC
7	0.86	yellow-green	brown	blue-white	HC
8	0.92	yellow-green	brown	red	PP, HC, CR

Notes: *Pennisetum purpureum* S. (PP); *Imperata cylindrica* L. (IC); *Hedyotis corymbosa* L. (HC); *Cyperus rotundus* L. (CR)

Table 4: Antibacterial activity

Plant	Extract concentration (mg/ml)	Diameter of Inhibition (mm)			
		ATCC	Isolate 1	Isolate 2	Isolate 3
P. purpureum S.	500	11.6±0.06	13.9±0.06	14.9±0.02	13.9±0.14
	400	11.9±0.01	13.2±0.06	14.1±0.06	13.2±0.21
	300	12.0±0.00	12.7±0.12	11.9±0.12	12.1±0.10
	200	12.0±0.10	12.0±0.00	11.1±0.11	12.0±0.00
I. cylindrica L.	500	14.0±0.00	14.9±0.16	16.1±0.14	13.0±0.21
	400	15.0±0.08	14.1±0.03	15.1±0.21	12.9±0.14
	300	14.1±0.04	13.1±0.12	14.9±0.21	12.9±0.14
	200	12.9±0.13	11.2±0.12	13.1±0.16	11.3±0.06
H. corymbosa L.	500	13.2±0.01	13.0±0.00	12.8±0.02	12.1±0.06
	400	12.4±0.02	12.2±0.01	11.6±0.10	11.9±0.06
	300	11.5±0.05	11.9±0.01	11.5±0.00	11.4±0.28
	200	11.2±0.02	11.5±0.02	10.9±0.07	11.9±0.21
C. rotundus L.	500	11.4±0.02	12.7±0.02	12.6±0.28	14.0±0.06
	400	12.1±0.01	12.2±0.00	12.2±0.21	13.9±0.14
	300	11.4±0.20	12.1±0.03	11.3±0.14	13.8±0.21
	200	11.0±0.00	12.0±0.01	11.0±0.14	13.0±0.28

*diameter of perforator = 9 mm

The resulted anti-shigellosis showed that all weed extracts produced higher inhibition to sensitive than resistant strains, shown in table 4. However, the resulting inhibition zones against all resistant isolates lead all weed extracts as a natural anti-shigellosis with a wide-spectrum and can be prospected to overcome the resistance cases of the disease.

The MIC is interpreted as the endpoint concentration of the extract in the first tube where bacterial suspension appears as a clear solution visually, compared to negative control which only contain MHB sterile without extract or bacterial inoculum. But, when the tubes representing the MIC at least two of the more tubes are sub-cultured and enumerated which is the lowest concentration showing no colony growth or 99.9% of the original colonies was killed, then this concentration is termed as the MBC value of the extract. The MBC test determines the lowest concentration at which an antimicrobial agent will kill a particular microorganism. In this study, the MIC-MBC values of each weed on sensitive and resistant bacterial strains, respectively, were as follow: *P. purpureum* S (S=≥12.5; R≧25 mg/ml); *I. cylindrica* (S=≥50.0; R≧ 25-100 mg/ml); *H. corymbosa* (S=≥25; R=≥25-100 mg/ml); and *C. rotundus* (S=≥25-50; R=≥50-100 mg/ml), presented in table 5.

Table 5: MIC and MBC values

Plant	MIC (mg/ml)				MBC (mg/ml)			
	ATCC	S1	S2	S3	ATCC	S1	S2	S3
P. purpureum S	12.5	25.0	25.0	25.0	12.5	25.0	25.0	25.0
I. cylindrica L.	50.0	50.0	50.0	20.0	50.0	25.0	25.0	100.0
H. corymbosa L.	25.0	50.0	100.0	100.0	25.0	25.0	50.0	50.0
C. rotundus L.	50.0	100.0	100.0	100.0	25.0	50.0	50.0	50.0

Notes: ATCC= sensitive strain; resistant strain isolate against: ampicillin (S1), chloramphenicol (S2), and cotrimoxazole (S3)

DISCUSSION

The *S. dysentriae* isolates used in this study were used because they are related with the source of gastrointestinal infections. Moreover, the effect of the extracts would describe their potent as effective and natural anti-shigellosis to overcome all isolate *S. dysentriae* included the resistant bacteria. The result of this study considered to be important, in the light of concurrent with the increased case of *S. dysentriae* resistance to antibiotics. In Indonesia, *S. dysenteriae* have been reported to be resistant against ampicillin (82%), cotrimoxazole (84%), and chloramphenicol (82%) [8]. In the US, Shigella's resistance rate to fluoroquinolones was 87% during 2014-2015 [27]. In most of the world, several Shigella strains have now been resistant to several drugs with various mechanisms and these mechanisms pose limitations of therapeutic options for shigelosis [28, 29]. Mutation or absence of ~39 kDa porin in the membrane of Shigella spp. mainly influences susceptibility to slow penetration of β-lactams [30, 31]. Resistance *S. dysentriae* to chloramphenicol was related mainly with the activity of Chl acetyltransferase [32]. The resistance of Shigella isolates to fluoroquinolones is mainly due to mutational changes in the QRDRs DNA gyrase and topoisomerase IV genes, but PMQR may facilitate the selection of isolates that exhibit higher levels of resistance through extra-chromosomally encoded mechanisms and reduced susceptibility to quinolones (or fluoroquinolones) [33]. To date, there has been a corresponding decline in antimicrobial discovery [6, 7, 9-12]. The alternative treatment strategy is important to be developed and considered by WHO to be the greatest challenge facing medicine [34, 35]. This has lead researchers toward alternative drugs, including traditional plant-based medicines and combinational therapies [36].

In this study, inhibition of *S. dysentriae* by agar diffusion method: all weed extracts showed that these extracts provided antibacterial activity, which was supported by the discovery of antibacterial compounds in the extracts. Secondary metabolite compounds produced by plants, not only function for their primary metabolism, but are also needed to adapt plants to adverse environments [37]. During evolution, the structure of these secondary metabolites has been optimized so that they can contribute to the plant defense system by inhibiting microbial molecular targets [38]. The phytochemical content in various plant extracts is able to inhibit protein-protein interactions leading to certain modifications. These modifications affect the process of microbial pathogenicity, even leading to microbial death. Thus, the diversity of compounds contained in these plant extracts can interact with protein domains in microbes so that they can reduce potency the resistance developed by microbes [39]. The response of the tested bacteria to each extract can be said to be different. In general, all extracts were more effective at inhibiting ampicillin-resistant *S. dysentriae* isolates compared to other resistant antibiotics, thus acting as a broad spectrum. *P. purpureum* and *C. rotundus* extract were potent to inhibit the resistant strain than the ATCC, however *H. corymbosa* extract revealed to provide higher antibacterial activity against the ATCC strain than the resistant. Among of the extracts, *H. corymbosa* extract demonstrated the highest inhibition against isolate 2 (chloramphenicol-resistant bacteria). From these various result towards the resistant bacteria, we can hypothesize that the phytochemical substances of each extract have main bacterial resistant target to inactivate or to inhibit and possibly related to the content of antibacterial phytochemical compounds in each extract. Of course, these results could provide an important contribution to replace or complete the shigellosis treatment considering the the extended use of antibiotics has led to drug resistance. Thus, all these weeds ethanolic extracts have the potential to complement antibiotics that are no longer effective in the treatment of shigella infections. The phytochemicals detected in these extracts have been reported to inhibit the growth of S*. dysentriae*. The results of phytochemical analysis showed that the extract contained different phytochemicals that included flavonoids, saponins, polyphenol, steroid, and quinones. Phytochemical screening describes the content of active substances in the extract while the number and properties of active substances can be identified efficiently based on TLC. The number of substances found with the same eluent will be different for each extract. It was reported that alkaloids, flavonoids and phenol compounds could be successfully detected in different extracts using TLC [39]. Similarly, in another study, the TLC profile of *Euphorbia thymifolia* extract could detect several good-quality flavonoid compounds [40]. It is affected by the difference in the polarity level of the phytochemical substance. This phenomenon is in accordance with the principle of like dissolved like, where polar substances can be attracted to polar solvents and vice versa. The visible colored spots exhibit the presence of chemical substances dissolved by the eluent used. The profile TLC, as displayed in table 2, showed the compounds separated by n-hexane: ethyl acetate (40: 60) as the non-polar mobile phase. There were four spots with the same Rf value and color in each weed extract. Based on TLC results, it is concluded that there are at least four relatively nonpolar compounds contained in each extract. The chromatography results in table 3 showed the compounds separated by ethyl acetate: methanol (60: 40) as the polar mobile phases. There were four spots with Rf and the same color in some extracts. So, it can be concluded that there are at least four relatively polar compounds in the extract of *H. corymbosa* and *P. purpureum*, three compounds in the extract of *C. rotundus*, and two in the extract of *I. cylindrica.* Based on the polarity, it can be assumed that the polar compounds referred to in the TLC results were flavonoids, polyphenols, quinones and tannins. The hydroxyl group of flavonoids makes it easily soluble in polar solvents but insoluble in non-polar compounds. Similarly, polyphenols are categorized as polar compounds because of the

presence of glycosides, namely sugar bonds with phenolics in cell vacuoles [41]. Those substance have been reported their efficacy as antimicrobial and resistance modifiers [42]. Among those weed extracts, *P. purpureum* provide the most complete antibacterial phytochemicals. This fact was related to its stronger antibacterial activity than other extracts. Flavonoids are phytochemical compounds that have been shown to have a broad antibacterial spectrum with different mechanisms [43-46]. Several studies had been reported various antibacterial mechanism of flavonoids including the inhibition of nucleic acid synthesis, interfere the function of cytoplasmic membrane and energy metabolism, reduce bacterial adhesion to form biofilm, interrupt porin, and reduce membrane permeability [47-51]. Flavonoids have a broad antibacterial spectrum against various bacteria by acting on microbial cell membranes by interacting with membrane proteins present in bacterial cell walls [52-56]. Phenolic substances have been reported to have antibacterial activity against Shigella [57]. Tannins extracts also have been reported to inhibit biofilm formation of *S. dysenteriae* and inactivate transport protein on cell envelopes and bacterial adhesin [58-61]. From the mode of action, tannins compounds are very important considering that recently, much attention has been paid to biofilm formation in bacteria, as microbial cells grown in biofilms are less sensitive to antimicrobial agents and more resistant to environmental stressors such as dehydration and oxidation. Microbe infection caused by Shigella spp. is a challenge for the world of health [62]. While the antibacterial mechanism of alkaloids occurs by intercalating DNA, which inhibits bacterial cell division and cell death [63]. Alkaloids such as cryptolepine and quindoline from *Sida acuta* are reported to be active against *S. dysenteriae* [64]. The targets of quinones are adhesin proteins located on the cell surface, polypeptides on the cell wall, and membrane-bound enzymes [65]. Then the mode of antibacterial action of saponins is focused on the decreasing permeability of bacterial membrane cells [66-70]. Several important function of those secondary metabolites found in the weed extracts makes bacteria unable to form resistance properties easily.

MIC is considered a standard value for assessing the susceptibility of organisms to antimicrobials. The MIC values obtained can confirm the limited resistance of bacteria if other methods are used or the results of the diffusion method are not suitable [68]. In the concentration range of 20 to 1.25 % w/v, the growth of all strains *S. dysentriae* was inhibited. MBC test results demonstrated that at a range concentration of 10 to 1.25 % w/v, 99.9% of the tested bacteria were killed. Among on those MIC-MBC data, *P. purpureum* has the largest anti-shigellosis potential with the smallest MBC value among all the test weeds extracts. However, the ratio of MBC/MIC values of all tested extracts against sensitive and four resistant *S. dysentriae* isolates were ≤4; thus, all extracts may be classified as bactericidal agent [72, 73].

CONCLUSION

Our findings revealed that the weeds extract used in this study provide effective treatment modalities to face *S. dysentriae* resistant to conventional antibiotic treatments. Therefore, the findings of the extract's ability to inhibit *S. dysentriae* resistant has become a novelty in the discovery of anti-shigellosis drugs, which in the future can be further investigated to overcome the resistance of other bacteria to the same antibiotics.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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