

THE PHYTOCHEMICAL SCREENING HEXANE AND METHANOL EXTRACT OF SINYO NAKAL (*DURANTA REPENS*)

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ABSTRACT

Objective: Phytochemicals are naturally present in every part of plants range from leaves, stem bark, and fruits to roots. The use of plant-based medicine is popular among individuals and communities in developing countries. This study evaluated that the phytochemical profile of *Duranta repens* fruits was collected from Jombang, East Java, Indonesia.

Methods: Air-dried *D. repens* fruits were macerated by *n*-hexane and methanol, a separate flask. *n*-Hexane extract, then underwent saponification to remove fat. Then, the unsaponifiable of *n*-hexane and methanol extract was tested through phytochemical screening, respectively.

Results: The methanol extract showed the presence of alkaloids, flavonoids, tannins, and steroids, but the unsaponifiable fraction only contained steroids. The unsaponifiable fraction was found to be a mixture of hydrocarbons ranging from C₁₃-C₂₀, fatty acids (palmitic and linoleic acid), squalene, and Vitamin E.

Conclusion: The phytochemical screening of the plant plays an important role for pharmaceutical studies especially discovering new potential drugs for the treatment of various diseases.

Keywords: *Duranta repens*, Unsaponifiable hexane fraction, Methanol extract.

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INTRODUCTION

The use of medicinal plants has become popular among individuals and communities in developing countries. Plants produce various bioactive compounds that contain components of therapeutic value making them a rich source of medicines. *Duranta repens* Linn. belonging to family *Verbenaceae* is native to clean and open forests. It is used as an ornamental plant in tropical nations. The local community in Indonesia commonly calls this plant as sinyo nakal (Fig. 1). The plants belong to *Verbenaceae* family which are herbs, shrubs, or trees comprising about 100 genus and 2600 species [1]. Around 35 *Duranta* species with evergreen bushes are spread over tropical and subtropical areas. *D. repens* is also known as *Duranta plumieri*, *Duranta erecta*, and *Duranta microphylla* [2,3].

Different parts of the plant are used to treat a variety of diseases. The fruit and leaves are used in traditional folk medicine for the treatment of malaria [4], and anti-shigellosis cytotoxic potency [5]. Methanolic extracts of different parts such as leaves, stem, and roots of *D. erecta* exhibited antifungal properties against *Aspergillus flavus*, *Alternaria* sp., *Penicillium* sp., *Rhizopus* sp., and *Trichoderma* sp., [6]. The ethyl acetate extract of leaves reportedly exhibited significant antiplasmodial activity against the chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* [7]. Fruits and stems of *D. repens* exhibited antifungal and antibacterial properties, cytotoxic, and larvicidal to *Culex quinquefasciatus* [8-10]. Leaves of *D. repens* exhibited antibacterial properties [4], antioxidant [11], and plant growth-inhibiting activity against seedlings of *Brassica juncea* var. *cernua* [7]. The ethyl acetate fraction of the methanol extract of the whole plant of *D. repens* showed radical scavenging activity in the 1,1-diphenyl-2-picrylhydrazyl assay [12], antiviral [13], insecticide properties against *Aedes aegypti* and *Attagenus piceus* [14], enzyme-

inhibitory activity against thrombin [15], and α -glucosidase Inhibitory [16].

The pharmacological significance was noted due to the presence of various bioactive compounds. Several bioactive compounds such as β -sitosterol, naringenin, acteoside, lamiide, sucrose, and raffinose have been identified and isolated from *D. repens* [13]. Plant of *D. repens* is reported to produce triterpenoid-type saponins [7], *C*-alkylated flavonoids [15,16], diterpenoids [15], phenylethanoid glycoside acteoside, the iridoid lamiide and the saponin pseudo-ginsenoside-RT₁ [12], coumarinolignoids [7,14,17], flavonoid and flavonoid glycosides [17], triterpenoids [18], triterpene saponins [19], iridoid glycosides [20], and steroids [21].

Even though many publications have reported the constituents of *D. repens*, this plant from Indonesia has still limited and never been reported, yet. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, and seeds. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for the synthesis of complex chemical substances. Therefore, the current study was aimed to carry out the phytochemical screening of *D. repens* grown in Jombang, East Java, Indonesia, as a justification for its use in traditional medicine. The difference in geographical location will affect the presence of secondary metabolites and drives our interest to conduct the phytochemical investigation of fruits of *D. repens*.

METHODS

Collection of plant materials

Fruits of sinyo nakal (*D. repens*) were collected from trees in Kabupaten Jombang, East Java, Indonesia.

Preparation of sample

The collected fruits were cut into tiny pieces, cleansed, and oven-dried in below temperature 40°C for 1–2 weeks. The fruits were ground into a powder and stored in sealed containers to avoid contamination and spoilage.

Preparation of extracts

Five hundred grams of dried powder of *D. repens* fruits were packed in a separate round bottom flask for sample extraction using *n*-hexane and methanol as solvent, respectively. The extraction was conducted by 750 mL of the solvent for 72 h or till the solvent in the siphon tube of an extractor become colorless. Saponification was then performed to the *n*-Hexane extract NaOH 0.5 N. The saponification process removed fat or oil or lipid in hexane extract and left an insoluble fraction, known as unsaponifiable fraction. Then, the unsaponifiable fraction and methanol extract were concentrated under reduced pressure and the dried extract was kept in the refrigerator at 4°C for their future use in phytochemical analysis.

Phytochemical analysis

The extracts were prepared for analysis of its phenol, tannins, saponins, alkaloids, flavonoids, terpenoids, and steroids content based on the protocols [22].

Test for phenols and tannins

The crude extract was mixed with 2 mL of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for saponins

The crude extract was mixed with 5 mL of distilled water in a test tube, and it was shaken vigorously. The formation of stable foam was taken as an indication of the presence of saponins.

Test for alkaloids

The extracts of *D. repens* fruit were evaporated to dryness in a boiling water bath. The residues were dissolved in 2 N hydrochloric acids. The mixture was filtered, and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, one portion was treated with an equal amount of Dragendorff's reagent, and the third portion was treated with an equal amount of Wagner's reagent, respectively. The appearance of a white precipitate, the orange precipitate, and brown precipitate indicated the presence of respective alkaloids. Meyer's reagent was prepared by measuring 1.3 g of mercuric chloride on a weighing balance and 5.0 g of KI (potassium iodide) and dissolving both in distilled water, made up to 100 mL. Wagner's reagent was prepared by measuring 2 g of iodine and 6 g of KI (potassium iodide) on a weighing balance and dissolving both in distilled water and made up to 100 mL. Dragendorff's reagent was prepared by measuring 5.2 g Bismuth carbonate and 4 g potassium iodide on a weighing balance and dissolving both in 50 mL glacial acetic acid and then boiled for a few minutes. After 12 h, precipitated sodium acetate crystals are filtered by sintered glass funnel, 40 mL filtrate was added 160 mL ethyl acetate and 1 mL distilled water, then stored in an amber-colored glass bottle. 10 mL stock solution was added to 20 mL acetic acid and dissolving in distilled water to make a final volume of 100 mL.

Test for flavonoids (shinoda test)

The extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added dropwise. The pink scarlet color appeared after a few minutes, which indicated the presence of flavonoids.

Test for steroid

The extract was mixed with 2 mL of chloroform. Then 2 mL of each of concentrated H₂SO₄ and acetic acid was poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for terpenoids

The extract was dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H₂SO₄ was added and

heated for about 2 min. A grayish color indicated the presence of terpenoids.

RESULTS AND DISCUSSION

Qualitative phytochemical screening of fruits of *D. repens* indicated the presence of tannins, saponins, flavonoids, alkaloids, and steroids for methanol extract, but only steroids for unsaponifiable fraction (Table 1). The unsaponifiable fraction was found to be a mixture of hydrocarbons ranging from C₁₃-C₂₀, fatty acid (palmitic and linoleic acid), squalene (1), and tocopherol (Vitamin E, 2), while from methanol extract has been elucidated as *trans*-cinnamic acid (3) by NMR includes ¹H, ¹³C, HSQC, HMBC, and TOCSY spectrum [23]. The compounds structure of 1, 2, and 3 is illustrated in Fig. 2.

A previous study [13] reported that the unsaponifiable fraction of whole parts of *D. repens* that was collected from Giza, Egypt, contained a mixture of hydrocarbons ranging from C₁₅-C₂₇. Another report [18] explained that chemical investigation of the dichloromethane extract of the flowers of *D. erecta* L. from the Philippines has led to the isolation of oleanolic acid, a mixture of α -amyirin and β -amyirin in a 3:1 ratio, phytol fatty acid esters, and triacylglycerols.

Literature studies by Ahmad et al. [14] reported phytochemicals contain in the methanol extract of *D. repens* leaves that were collected around Mysore, India, included tannins, saponins, flavonoids, glycosides, steroids, and terpenoids. Phytochemical profiling of *D. repens* also revealed the presence of some imperative phytochemicals such as alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and terpenoids [1]. Phytochemical screening of the ethanol extract of *D. erecta* leaves and fruits from Ghana also indicated the presence of tannins, glycosides, saponins, flavonoids, triterpenoids, and sterol in ethanol extract leaves, both unripe and ripe fruit. Besides, unripe fruit also contained alkaloids [24]. The methanol extract of *D. erecta* leaves that were collected from Tamil Nadu, South India, was found to contain sugars, tannins, alkaloids, phenols, flavonoids, saponins, triterpenes, and carboxylic acid. Moreover, the aqueous extract was found to contain tannins, alkaloids, phenols, flavonoids, saponins, catechins, and glycosides [10].

The methanol extract of *D. erecta* (L) leaves that were collected from CSIR-National Chemical Laboratory Colony, Pune, indicated the presence of secondary metabolites such as glycoside, saponins, sterols, flavonoids, phenols, tannins, alkaloids, carbohydrates, and proteins [25]. The aqueous extract of *D. erecta* fresh leaves was collected from the residential quarters within the University of Nigeria, Nsukka, indicated small quantities (+) of alkaloids, flavonoids, tannins, anthraquinone, and glycosides in the extract, while saponins were moderately present (++) , steroids were conspicuously absent [26]. The 70% aqueous ethanol of *D. erecta* L. leaves was collected from Nigeria indicated the presence of secondary metabolites such as steroidal glycoside, saponins, flavonoids, polyphenols/tannins, alkaloids, and terpenes [27]. The difference in phytochemicals results was presumably due to several factors, such as growing area, environmental stress such as heavy metals or ultraviolet exposure, age of plants, genetic factors, and physical factors such as climate, humidity, temperature, and weather [28].



Fig. 1: Morphology of the plant (a) and (b) fruits of *Duranta repens* L.

Table 1: Phytochemicals screening of *D. repens*

Plant	Extract	Secondary metabolites					
		tannins	saponins	flavonoids	alkaloids	steroids	Terpenoids
<i>D. repens</i> fruits	MeOH	+	+	+	+	+	-
	Unsaponifiable fraction	-	-	-	-	+	-
<i>D. repens</i> leaves [14]	MeOH	+	+	+	-	+	+
<i>D. erecta</i> [24]							
Leaves	EtOH	+	+	+	-	-	+
Unripe fruits		+	+	+	+	+	+
Ripe fruits		+	+	+	-	+	+
<i>D. erecta</i> leaves [10]	MeOH	+	+	+	+	-	+
	Water	+	+	+	+	-	-
<i>D. erecta</i> leaves [25]	MeOH	+	+	+	+	+	-
<i>D. erecta</i> fresh leaves [26]	Water	+	+	+	+	-	-
<i>D. erecta</i> (L) leaves [27]	70% aqueous ethanol	+	++	+	+	+	+

D. repens: *Duranta repens*, *D. erecta*: *Duranta erecta*

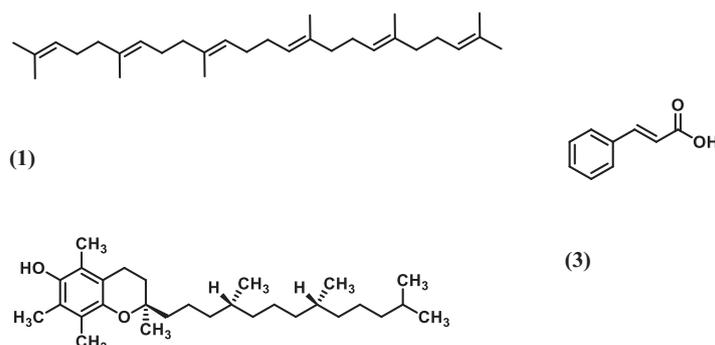


Fig. 2: The structures of (1) and (2) from unsaponifiable fraction and (3) from methanol extract of *Duranta repens* fruits were collected from Jombang, East Java, Indonesia

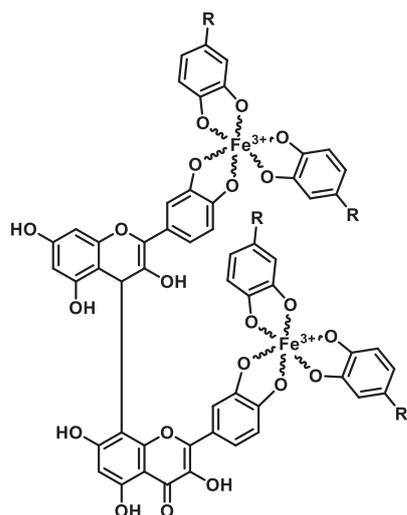


Fig. 3: The proposed tannin complex with $FeCl_3$

In tannin testing, methanol extract showed positive results with the formation of a blackish green color that indicates the formation of complex compounds between tannins and Fe^{3+} ions, but not detected in unsaponifiable fraction. The blackish-green color has been known to be the complex between Fe^{3+} and tannins (polyphenols) [29]. The reaction between tannic acid and $Fe(III)$ in carbonate buffer solution forms yellow-green complex while the reaction between tannic acid and $Fe(II)$ in carbonate buffer solution forms magenta complex. The tannin acts as a ligand due to the presence of O atom, which has a pair of free electrons that can bind covalent coordinates to the Fe^{3+} as the central ion. Fig. 3 explained that Fe^{3+} ions bind to three pairs of oxygen donor at 4' and 5' positions from the tannins structure. This position has the lowest energy, thus allowing to form a stable complex.

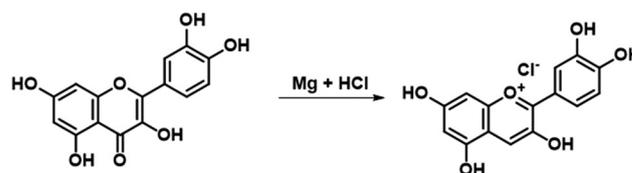


Fig. 4: The proposed flavonoids reaction with Mg and HCl

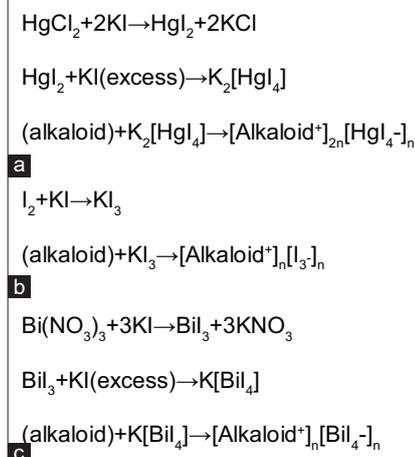


Fig. 5: The proposed alkaloid reaction with Mayer's (a), Wagner's (b), and Dragendorff's (c) reagents

In flavonoids testing, methanol extract showed positive results with the formation of a dark yellow to red solution that indicating the formation of flavylum salts. In contrast, unsaponifiable fraction suggested to no

flavonoid content. The reaction of flavonoids with metals Mg and HCl has been known as the Willstatter and Bate-Smith reactions. Metal Mg that was involved in the reaction acts as a reducing agent which transforms the benzopyrone moiety in the flavonoid structure to red or orange flavylum salts. The reaction refers to Clemmensen reduction. The metal supplies the electrons and the hydrochloric acid supplies the protons to achieve the carbonyl reduction. The proposed flavonoids reaction with Mg and HCl is explained in Fig. 4. This result was also confirmed to the reported result of flavonoid contents in *D. repens* from Pakistan and Egypt [13,16,17].

Alkaloid detecting reagents were solutions of the salts of heavy metals. The most common reagents are Mayer's (potassium iodide-mercuric chloride), Wagner's (iodine-potassium iodide), and Dragendorff's (potassium iodide-bismuth nitrate). In the preparation of Dragendorff's reagents, bismuth nitrate was dissolved in HCl to prevent the hydrolysis reactions due to bismuth salts were easily hydrolyzed to bismutyl ions (BiO^+). The reaction mechanism of action has been proposed to occur through coupling of the reagent's heavy metal atom in the reagent with the nitrogen in the alkaloid to form ion pairs. The ion pairs form an insoluble precipitate. The proposed alkaloid reactions with Mayer, Wagner, and Dragendorff reagents afforded precipitations white color complexes ($[\text{alkaloids}^+]_{2n}[\text{HgI}_4^-]_n$), light brown ($[\text{alkaloids}^+]_n[\text{I}_3^-]_n$), and orange ($[\text{alkaloids}^+]_n[\text{BiI}_4^-]_n$), respectively [30,31]. The precipitation color could vary from orange-red, yellow-orange, red-black, and pink-purple depending on the species or genus [32]. The proposed alkaloid reaction with all three reagents is explained in Fig. 5.

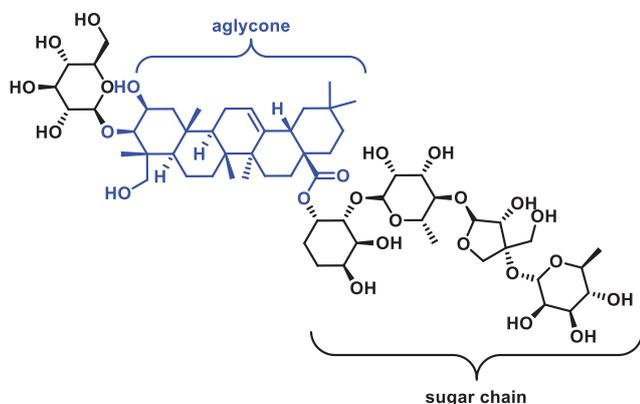


Fig. 6. Structure of Durantanin I, saponin from the leaves of *D. repens* leaves collected from Bangkok, Thailand

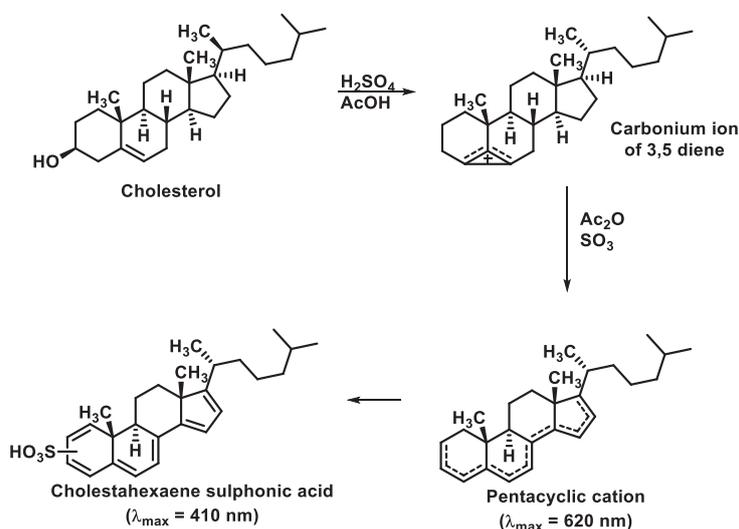


Fig. 7: Reaction scheme for derivatization of cholesterol through Liebermann-Burchard reaction

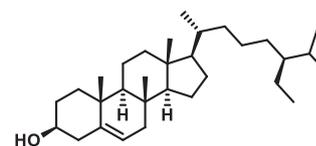


Fig. 8: Chemical structure of β -sitosterol

D. repens methanol extract also showed positive results on saponin identification. It formed a stable foam for 30 min, but the same result could not be detected in unsaponifiable fraction of *n*-hexane extract. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The foaming ability of saponins was caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part [33]. The previous study has also reported saponin content from *D. Repens* grown in Thailand and Egypt [7,19]. Durantanin I (Fig. 6) was the common saponins found in *D. repens* [17].

Moreover, both samples give a negative result in terpenoids testing, while steroids testing of both samples showed positive results which, as indicated by the formation of a bluish-green solution. Fig. 7 illustrated an example of the reaction of cholesterol with Liebermann-Burchard reagent [34]. The steroid core structure was typically composed of 17 carbon atoms, bonded in four "fused" rings: Three six-member cyclohexane rings (rings A, B, and C) and one five-member cyclopentane ring (the D ring). A steroid from ethanol extract of whole *D. repens* that was collected in Giza, Egypt, was identified as 24-ethylcholest-5-en-3- β -ol (β -sitosterol, 20) (Fig. 8) [13].

The phytochemical screening of *D. repens* plays an important role in pharmaceutical studies, especially to discover some new potential drugs for the treatment of various diseases. Therefore, *D. repens* extract could be a good source of multi-targeted drugs. The traditional medicine practice was recommended strongly for *D. repens* as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of this plant. The additional work is also encouraged to elucidate the mechanism that corresponds to the bioactivity of this extract.

CONCLUSION

The methanol extract of sinyo nakal fruits (*D. repens* L.) was the source of the secondary metabolites ranging from tannins, saponins, alkaloids, flavonoid, and steroids. *n*-Hexane extract was found to be a mixture of hydrocarbons ranging from C_{13} - C_{20} , fatty acid (palmitic and linoleic acid), squalene, and Vitamin E while unsaponifiable fraction of *n*-hexane extract was also found to contain steroids.

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