# The phytochemical screening and thin layer chromatography results of *Jatropha gossypiifolia* seeds

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**ABSTRAK**

***Latar belakang:*** *Schistosomiasis saat ini masih menjadi masalah kesehatan di daerah endemis di Sulawesi Tengah. Keong Oncomelania hupensis lindoensis, perantara schistosomiasis tersebar luas di wilayah tersebut. Pemberantasan yang dilakukan selama ini dilakukan dengan penyemprotan moluskisida kimia. Tujuan penelitian ini untuk mengidentifikasi golongan senyawa kimia di dalam ekstrak metanol biji jarak merah.*

***Metode:*** *Penelitian dilakukan pada bulan Mei 2009. Biji jarak merah dikumpulkan dari wilayah Tondo, Kota Palu. Ekstraksi biji jarak merah dilakukan dengan metode perkolasi menggunakan pelarut metanol. Dilakukan skrining fitokimia dengan uji tabung untuk mendeteksi golongan senyawa di dalam ekstrak biji jarak merah. Skrining dilanjutkan dengan uji kromatografi lapis tipis untuk memastikan hasil skrining dari uji tabung.*

***Hasil:*** *Ekstrak yang dihasilkan dari 500 gram serbuk biji jarak merah kering dengan 2500 ml pelarut metanol adalah sebanyak 250 ml ekstrak kental berwarna coklat kemerahan. Skrining fitokimia dengan uji tabung menunjukkan hasil positif alkaloid dengan terbentuknya endapan pada reaksi tes Meyer, tes Wagner, tes Dragendorff. Skrining ekstrak metanol biji jarak merah juga menunjukkan hasil positif saponin pada tes busa dan tes Lieberman-Burchard. Hasil positif pada tes Keller Killiani dan tes Kedde menunjukkan bahwa ekstrak biji jarak merah mengandung cardenoline dan bufadienol. Analisis kromatografi lapis tipis menunjukkan ekstrak biji jarak merah positif terpen dengan terbentuknya spot pada plat silika gel dengan penyemprotan pereaksi terpen yaitu serium sulfat.*

***Kesimpulan:*** *Komponen kimia yang terkandung dalam ekstrak metanol biji jarak merah terdiri dari alkaloid, saponin, cardenolin, bufadienol, dan terpen.* ***(Health Science Indones 2012;2:xx-xx)***

***Kata kunci:*** *schistosomiasis, Jatropha gossypifolia, komponen kimia*

**ABSTRACT**

**Background:** Schistosomiasis is still a health problem in Central Sulawesi. Snail *Oncomelania hupensis lindoensis*, the intermediary for schistosomiasis is widespread in this region. Eradication has been done by spraying chemical molluscicides. This study aimed to identify the class of chemical compounds in the methanol extract of red castor seed.

**Methods:** The study was conducted in May 2009. Red castor seeds were collected from Palu, Central Sulawesi. Red castor seeds extraction was done by percolation method using methanol solvent. Phytochemical screening test was performed with a tube to detect the compound in red castor bean extract. Screening was followed by thin layer chromatography testing to ensure the screening results of the test tube.

**Results:** Extracts that was produced from 500 grams of red castor dry seed powder with 2500 ml of methanol solvent was 250 ml thick reddish brown fluid. Phytochemical screening with a test tube showed positive results of alkaloid by the formation of deposits in Meyer test, Wagner test, and Dragendorff test. Screening the methanol extracts of red castor seed also showed positive results on saponins by foam test and Lieberman-Burchard test. Positive results on Killiani Keller tests and Kedde test suggests that red castor bean extract contains cardenoline and bufadienol. Thin-layer chromatography analysis showed that the red castor bean extract is positive for terpenes with the formation of spots on the silica gel plate when terpenes was sprayed (cerium sulfate reagent).

**Conclusion:** Chemical components contained in the methanol extract of red castor beans consisted of alkaloids, saponins, cardenolin, bufadienol, and terpenes. ***(Health Science Indones 2012;2:xx-xx)***

**Keywords**: schistosomiasis, *Jatropha gossypifolia*, chemical compound

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Schistosomiasis, also called bilharziasis is still a public health problem in the world after malaria. It is estimated that more than 200 million people worldwide are infected by a worm that is transmitted by snail. Schistosomiasis is endemic in 74 developing countries, particularly in rural areas. It is now estimated that there are 650 million people living in endemic areas.[1]

Schistosomiasis or snail fever disease in Indonesia are known in the area of Lindu, Napu and Bada Highlands (Central Sulawesi). The case of the disease was first found by Muller and Tesch (1937). The intermediate host of schistosomiasis was found in 1971 and identified as *Oncomelania hupensis lindoensis.*[2]

Intermediate host or snail eradication efforts have been made by Schistosomiasis Control Program in Central Sulawesi Provincial Health Office through the spraying of molluscicides Bayluscide every 6 months.[3] The long term effects from the use of bayluscide needs to be studied at this point. Furthermore, the use of chemical molluscicides has the disadvantage that the material is more expensive and lead to greater pollution of the environment.[4]

There is a number of molluscicides plant to kill the snails from the *Jatropha* (*Jatropha curcas*) family Euphorbiaceae. Extract from plant seed has potential as molluscicides against *Oncomelania hupensis* and *Biamphalaria glabrata*, by LC100 value at 1 ppm.[5]

Research is also needed on other plants that are still one family with the plant that has potential of molluscicides that has been studied. In this research, phytochemical screening was conducted to identify the types of classes of chemical compounds contained in the extract of red castor seeds (*Jatropha gossypiifolia*).

*Jatropha gossypifolia* (synonym: *Adenoropium gossypifolia* Pohl, *Jatropha elegans*) is included in the family Euphorbiaceae. This plant is a shrub with a height of 1.8 meters, and its young leaves are colored red-purple.[6]

## METHODS

The red castor seeds (*Jatropha gossypiifolia*) were obtained from the District of Tondo, Palu (Central Sulawesi). Seeds were then sun-dried and covered with black cloth for 3 days. Once the seed is pulverized and dried, then methanol extract was made by way of percolation.

Materials used for the test tubes were several reagents and silica gel plate to identify the class of secondary metabolites.

### The red castor seeds (*Jatropha gossypiifolia*) extraction by percolation

The process of extraction and phytochemical screening was done at Galenika Laboratory, Center for Medicinal Plants and Traditional Medicine Tawangmangu (Central Java). Crude powder weighed as much as 500 grams and was then inserted into the vessel. The next step is moistening the powder with the solvent. The powder was stirred until it was smooth, then it was covered and let to stand for 3 hours within a place protected from sunlight.

The crude powder was then put into the percolator little by little, then flattened and placed on top of the filter paper. The next step was adding a solution of 2,500 ml methanol solvent and drip them on the percolator with a speed of 1 ml / min.

The next process was putting the powder in a percolator and allowing them to stand for 24 hours, followed by dropping them into the solvent and extract them simultaneously with a speed of 1 ml / min.

The obtained extract was then separated from the solvent in a vacuum rotary evaporator to get the viscous extract. Then it was evaporated and then condensed on water bath to remove residual solvent.

### The phytochemical screening

### Tube test

Tube test were done to detect the presence of alkaloids (introduction, alkaloids primary, secondary, tertiary amine oxide test), saponins (foam test, Liebermann-Burchard test), Cardenoline and Bufadienol (Keller-Killiani, Kedde), flavonoids (Bate-test Smith & Metcalf, cyanidin Wilstater test), tannins and polyphenols (gelatin test, test FeCl3), antraquinon (Borntragers test).

### Thin layer chromatography

This test was done to ensure the test results with test tubes on group of chemical compounds contained in red castor bean extract. Silica gel plate was eluted with a mobile phase (n butanol: acetic acid: water = 4:1:1).

Spots that formed on the silica gel plate was viewed under UV light with a wavelength of 254 and 365 nm.

## RESULTS

The extract obtained from 500 grams of red castor seed powder with 2500 ml of methanol solvent was 250 ml thick reddish brown fluid. Solvent extraction with methanol was done to take the polar components from the red castor beans sample. Tube Test Results on seed methanol extracts of red can be seen in table 1.

Table 1 shows the phytochemical screening's results with test tube / reaction of the color red castor bean extract. The chemical compounds found in red castor bean extract were alkaloids, saponins, and bufadienol cardenolin.

The silica gel plate was eluted with a mobile phase (n butanol: acetic acid: water = 4:1:1). Spots which formed on the silica gel plate was viewed under UV light with a wavelength of 254 and 365 nm. The spots indicates that the secondary metabolites contained in the methanol extract of castor beans are terpene compounds. This is evidenced by the formation of spots on the silica gel plate after thin layer chromatography (TLC) and the spraying of cerium sulfate which is a reagent for terpene compounds.

Tabel 1. The phytochemical screening on red castor seeds methanol extract (*Jatropha gossypiifolia*)

| Chemical compound | Test methods | Results | Notification |
|---|---|---|---|
| Alkaloid | Introduction | | |
| | Reagen Mayer | Light yellow | - |
| | Reagen Wagner | Turbid red | + |
| | Reagen Dragendorf | Turbid orange | + |
| | Ensuring | | |
| | Reagen Mayer | Light yellow | - |
| | Reagen Wagner | Turbid orange | + |
| | Reagen Dragendorf | Turbid | + |
| Saponin | Foam test | Stabil foam | + |
| | Test Lieberman Burchard | Purple | + |
| Cardenoline& Bufadienol | Tes Keller Killiani | Blue | + |
| | Tes Kedde | Purple | + |
| Flavonoid | Tes Bate Smith&Metcalf | No change | - |
| | Tes Wilstater sianidin | No change | - |
| Antraquinon | Tes Borntrager | No change | - |
| Tannin & Polifenol | +FeCl3 | No change | - |
| | +Gelatin | No change | - |

Remark: (+) = present, (-) = absent

## DISCUSSION

In this study group, the secondary metabolites found in the extract of red castor beans were alkaloids, saponins, cardenolin, terpenes and bufadienol. The results were consistent with several previous studies on the chemical content of the plant *Jatropha gossypifolia* extracts.

Selecting methanol as a solvent in this study was aimed to obtain the potential molluscicides compound, one of which is a terpene compounds. The use of methanol as a solvent in the extraction of castor seeds was able to draw red terpene compounds contained in red castor beans.

The formation of spots indicates that the secondary metabolites contained in the methanol extract of castor beans are terpene compounds. This is evidenced by the formation of spots on the silica gel plate after thin

layer chromatography (TLC) and after spraying cerium sulfate which is a reagent for terpene compounds

Terpenes are the largest class of chemical compounds that have broad biological activity. Plants that belongs to the genus Jatropha, family Euphorbiaceae which contains terpene compounds are quite many. Among the class of terpenes, diterpene compounds are the most dominant in terms of efficacy and biological activity of various terpene compounds.[7]

Diterpene compounds have been isolated from as many as 68 species of Jatropha, among which are rhamnofolane, daphnane, lathyrane, tigliane, dinorditerpene, deoxy preussomerin and pimarane.[7]

Diterpene compounds from the genus Jatropha has clear biological activities and are very varied. In general, the biological activity of diterpene compounds are cytotoxic, antitumor, antimicrobial, and anti-cancer. Phorbol ester diterpene which is included in the diterpenes class was reported having the biological activity of insecticides and molluscicides.[7]

The secondary metabolites contained in the *Jatropha gossypiifolia* plant were citlalitrione and jatrofenon (diterpenoid compounds), which were isolated from the whole plant *J. gossypifolia*. Jatrofenon have anti mollusks and antimicrobial activity against *Staphylococcus aureus* with activity comparable to penicillin G. The coumarin-lignoid compound which was also obtained as a whole is propasin.[8]

The formation of precipitate on the tests Meyer, Wagner and Dragendorff means the methanol extract of red castor beans contained alkaloids. The addition of HCl aims to neutralize the alkaline alkaloid, thus the need to add acid. The addition of NaCl before the addition of reagents Wagner aims to eliminate proteins that can cause precipitation that led to a false positive result on several compounds test.

Positive alkaloid results on Meyer tests are characterized by the formation of a white precipitate, which is thought to be a potassium complex - an alkaloid. Positive alkaloid results on Wegner tests are characterized by the formation of a brown to yellow sludge which is a complex of potassium - an alkaloid. Positive alkaloid results on Dragendorff tests is also characterized by the formation of potassium precipitate – a light brown to yellow alkaloid.

Alkaloid compounds are the most organic compounds found in nature. Almost all of the alkaloids are derived from plants and are widespread in many plant species. Organoleptically, leaves a bitter taste and sepat, are usually identified to contain alkaloids. In addition to leaves, alkaloid compound can be found in the roots, seeds, twigs, and bark.[9,10]

Based on the literature, it is known that almost all natural alkaloid have biological activity and specific physiological effects on living organisms. They have toxic properties and are useful for treatment. So far, the function of the alkaloid plant itself is not known for certain, some experts have suggested that they protect alkaloid plants from pests and diseases, function as plant growth regulators, or as a base mineral to maintain ion balance.[9]

Foam test to determine the presence of saponin glycosides have demonstrated the ability to form a stable foam in the water. Lieberman-Burchard test, which is a test for the characterization of the class of unsaturated sterols and triterpenes, was also performed to determine the existence of saponin.[11]

Saponin is a glycoside that exist in various kinds of plants. Saponins exist with particularly high concentrations in certain parts of plants, and is influenced by crop varieties and stages of growth. The function of saponin in plants is unknown, possibly as a storage form for carbohydrate, or as a waste product of plant metabolism. Another possibility is for protection against insects.[12]

Positive reaction for cardenoline and bufadienol is marked with the color change of brown color pink rings to blue or purple on the Killiani Keller test. Another positive reaction is by the formation of blue violet color on the Kedde test. Bufadienolid is a class of secondary metabolites from plants in the form of steroid compounds that are toxic, commonly found in the form of glycosides.[13]

In conclusion, the chemical components contained in the methanol extract of red castor beans consists of alkaloids, saponins, cardenolin, bufadienol, and terpenes.

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## REFERENCES


1. Schistosomiasis fact sheet. Geneve. WHO. 2009. [cited: 2009 October 11]. Available from: http://www.who.int.
2. Hadidjaja P. Schistosomiasis in Central Sulawesi, Indonesia. Jakarta. Universitas Indonesia. 1985:12. Indonesian.
3. The schistosomiasis prevalence in Central Sulawesi. Provincial Health Office Central Sulawesi. 2008. Indonesian.
4. Ruppei A, Rug M. Toxic activities of the plant *Jatropha curcas* against intermediate snail hosts and larvae of schistosomes. Trop Med International Health. 2000;5:423-30. [cited: 2006 September 7]. Available from : http://www.aseanbiodiversity.info/51009517.pdf
5. Liu SY, Sporer F, Wink M, *et al*. Anthraquinones in *Rheum palmatum* and *Rumex dentatus* (Polygonaceae), and phorbol esters in *Jatropha curcas* (Euphorbiaceae) with molluscicidal activity against the schistosome vector snails *Oncomelania*, *Biomphalaria* and *Bulinus*. *Trop Med and International Health* 2.1997:179–88.
6. Balee W. Footprints of forest. Ka'apor ethnobotany-the historical ecology of plant utilization by an Amazonian people. New York. Columbia Univ Press. 1994.
7. Devappa, Rakshit K, Makkar, *et al*. Jatropha toxicity—a review. Toxicol Environ Health. 2010;136:476-507. [cited: 2012 October 16]. Available from : http://dx.doi.org/10.1080/10937404.2010.499736
8. Das B, Venkataiah B. A rare diterpene from *Jatropha gossypifolia*. Biochemical systematic and ecology. 2001;29:213-4.
9. *Alkaloid*. 2009. [cited: 2009 December 6], Available from : http://id.wikipedia.org/wiki/alkaloid. Indonesian.
10. Lenny S. The flavonoid, phenilpropanoid, terpenoid, and alcaloid compound]. Universitas Sumatera Utara. 2006. [cited: 2012 October 16]. Available from: http://library_usu.co.id/download/fmipa/06003489.pdf. Indonesian.
11. Rusdi. Plant as the medicine recourses materials. Padang: Univ Andalas. 1990. Indonesian.
12. Oey KN. The toxic compound in vegetable food naturally. Cermin Dunia Kedokteran. 1989; 58: 24. [cited: 2009 December 6]. Available from : http://www.kalbe.co.id/files/CDK/files/58_10_zat_toksik_alamiah. Indonesian.
13. Cardenoline. 2009.[cited: 2009 December 6]. Available from: http://upload.wikimedia.org/wikipedia/commonsioloc/cardenoline. Indonesian.