Objective: The purpose of this research was to observe the bioactive compounds of Karo traditional oil by phytochemistry screening and gas chromatography-mass spectrometry (GC-MS) analysis.

Method: We use 150 mg sample of traditional Karo traditional oil, methanol, ethanol, Dragendorff reagent, acetic acid anhydride, sulfuric acid, HCl, magnesium powder, FeCl₃ 1%, KLT plates, test tubes, dropper pipette, porcelain cup, filter paper, and a boiler.

Results: Alkaloid, terpenoid and flavonoid compounds were found, and no polyphenol compound from phytochemistry screening and 16 constituents were identified from the GC-MS analysis, α-pinene was found as the major component (74.47%), delta-3-carene (9.62%), 1% of octane, dodecane, camphor, undecane, isododecane, sabinene, hexadecane, nonane, and +1% tridecane, cyclhexane, pentacosane, heptadecane, limonene, and camphene.

Conclusion: Karo traditional oil works as a potent anti-inflammation at the inflammation phase of the wound healing process by suppressing the pro-inflammatory cytokines and promote anti-inflammatory cytokines.

Keywords: Traditional medicine, Indonesia traditional medicine, Phytochemistry screening, Gas chromatography-mass spectrometry, Karo oil, Karo traditional oil, Wound healing.

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METHODS
Phytochemistry screening
In phytochemistry screening, we use 150 mg sample of traditional Karo traditional oil, methanol, ethanol, Dragendorff reagent, acetic acid anhydride, sulfuric acid, HCl, magnesium powder, FeCl₃, 1% KLT plates, test tubes, dropper pipette, porcelain cup, filter paper, and a boiler.

In alkaloid compound analysis, Karo traditional oil was diluted in 4 ml methanol and dropped 5 µm on KLT plate, and Dragendorff reagent dropped to the KLT plate. In terpenoid and steroid compound analysis, 50–100 mg Karo traditional oil sample is placed on the plate and dropped with acetic acid anhydride, left for about 15 min. Then, six drops of solution are transferred to the test tube and added 2–3 drops of concentrated sulfuric acid. The presence of a triterpenoid is indicated by the occurrence of orange or purple-red, while the presence of steroids is indicated by the presence of blue. In flavonoid compound analysis, 200 mg of plant samples were extracted with 5 ml ethanol and heated for 5 min in a test tube. Then, add a few drops of concentrated HCl Then, 0.2 g of magnesium powder is added. Positive results are indicated by the appearance of dark red (magenta) in 3 min. Analysis of saponin composition: Two grams of Karo traditional oil are put into a test tube so that the complete examination is submerged, boiled for 2–3 min, and then cooled, then shaken vigorously. Positive results are collected by the formation of a stable foam. Analysis of tannin composition: A total of 20 mg of traditional Karo traditional oil, plus ethanol until all samples are submerged. Then, 1 ml of the solution is transferred into the test tube, and 2–3 drops of 1% FeCl₃ solution are added. Positive results are considered with the formation of a black or green-bluish color.

GC-MS analysis
GC-MS analysis was performed on GC-MS Agilent technologies 6890N Network GC system, chromatographic column Agilent 19091-413 HP-S 30 m 0.32 mm ID (0.25 µm df) capillary, and Agilent technologies 5973 inert mass selective detector. The temperature program in profiling was 50°C in 5 min, then 280°C in 5 min, at 10°C/min. The carrier gas was helium 0.5 ml/min, the injector and detector temperatures were 250°C, injection of 10 ml hexane solution 10%.

The sample is extracted with 3 ml hexane, then poured into derivatization chamber and nitrogen is added. 2 ml NaOH 2% in methanol is added and then the solution is heated in 5 min. BF₃ in methanol is added then the solution is heated to 90°C within 30 min. Finally, 3 ml hexane is added and then vortex the solution for extraction. After all, let the solution stands until separated into two-phase. Take the upper phase to be injected into the GC-MS.

RESULTS
Phytochemistry screening result
In phytochemistry screening like shown on Table 1, we found that Karo traditional oil form is oil or viscous liquid with green color, its smell of mushroomy. In alkaloidal screening result positive, its reacted with the Dragendorff reagent formed orange, red sediment which is shown in Fig. 2a. Its terpenoid result is positive; there was a purple, red stain on the KLT plate which is shown in Fig. 2b. The flavonoid screening result with thin-layer chromatography method is positive, with a yellow stain on the KLT plate which is shown in Fig. 2c. The polyphenol and tannin screening results are negative because there was no blackish-brown stain on the KLT plate which is shown in Fig. 2d and e, and the saponin screening result is negative with no foam on the water surface which is shown in Fig. 2f.

GC-MS analysis result
In GC-MS screening test like shown on Table 2.

DISCUSSION
The recipe of traditional Karo oil comes from a mixture of 51 Indonesian family medicinal plants (TOGA plants) [4,6,7], some of them are leaves and the roots of Piper betle, Gymnobogon nardus, Avicennia marina, Solanum verticillowid, Sonchus arvensis', Kaempferia galangal L., Drymoglossum piloselloides L., Clerodendrum sp., Plantago major L., Imperata, Orthosiphon spicatus B.B.S., Strobilanthis crispa, Vitex trifolia L., Gymura procumbens, Gymura segetum, Vitis gracilis bl., Chromolaena odorata, Zingiber officinale Rosc., Pluchea indica, Areca catechu, Myristica fragrans, Piper nigrum, Zingiber purpureum Roob., Kaempferia galanga, Bambusa vulgaris Schard, Ulmus lancaefolia, Curcuma domestica Val, Imperata cylindrica, Macodes peto Bl., Arenga pinnata, Ocimum citriodorium Vis, Allium cepa, Allium sativum, Boehmeria nivea, Citrus hystrix, and Usnea barbara fries. All of these medicinal plants were cut and boiled with Green Coconut Oil (Cocos nucifera linn) and cooking oil for 9 h on the small firewood [4,8].

The phytochemical screening method is carried out by looking at the color testing reaction using a color reagent. The reasons to do phytochemical screening are to reveal the potential of plant resources...
Table 2: Karo traditional oil’s contents based on gas chromatography-mass spectrometry test result

| Compound         | Percentage | Molecular formula | Molecular weight (g/mol) | Peak area (%) | 3D structure |
|------------------|------------|-------------------|--------------------------|--------------|--------------|
| Alpha-pinene     | 74.47      | C_{10}H_{16}      | 136.23                   | 72.13        |              |
| Delta-3-carene   | 9.62       | C_{10}H_{16}      | 136.23                   | 9.32         |              |
| Octane           | 1.813      | C_{8}H_{18}       | 114.23                   | 3.40         |              |
| Dodecane         | 1.776      | C_{12}H_{26}      | 170.33                   | 1.72         |              |
| Camphor          | 1.766      | C_{10}H_{16}O     | 152.23                   | 1.71         |              |
| Undecane         | 1.162      | C_{11}H_{24}      | 156.31                   | 1.13         |              |
| Isodecane        | 1.093      | C_{10}H_{22}      | 142.28                   | 1.06         |              |
| Sabinene/thujene | 1.061      | C_{10}H_{16}      | 136.23                   | 1.03         |              |
| Hexadecane       | 1.023      | C_{16}H_{34}      | 226.44                   | 0.99         |              |
| Nonane           | 1.012      | C_{9}H_{20}       | 128.25                   | 0.98         |              |
| Tridecane        | 0.992      | C_{13}H_{28}      | 184.36                   | 0.96         |              |
| Cyclohexane      | 0.886      | C_{6}H_{12}       | 84.16                    | 0.86         |              |
| Pentacosane      | 0.878      | C_{25}H_{52}      | 352.7                    | 0.85         |              |
| Heptadecane      | 0.870      | C_{18}H_{36}      | 254.5                    | 0.84         |              |
| Limonene         | 0.940      | C_{10}H_{16}      | 136.23                   | 0.81         |              |
| Camphene         | 0.738      | C_{10}H_{16}      | 136.23                   | 0.71         |              |
and determine the characteristics of active compounds that cause toxic effects or beneficial effects, which are shown by the effects of coarse plants when tested with biological systems. The important thing that plays an important role in the phytochemical ring is the selection of solvents and extraction methods [9].

Alkaloids are secondary or tertiary or sometimes quartery nitrogen atom compounds found in plants. They are found in the form of water-soluble salts such as citrate, malate, meconate, tartrate, isobutyrate, benzoate, or sometimes in combination with tannins in plants. Microchemical, alkaloids were found in many peripheral tissues of the stem or roots and synthesized in specific locations such as growing roots, chloroplasts, and lactiferous cells [10]. Mayer or Dragendorff solutions are the reagents which are used to identify alkaloid compounds in a sample.

Terpenoids are a natural compound formed by biosynthetic processes, widely distributed in the cytoplasm of plant cells. They are found not only in higher plants but also in coral reefs and microbes. The terpenoid structure is fat-soluble and constructed by isoprene molecules, terpenoid skeletons are formed from two or more units of isoprene units. In this research, terpenoids are the dominant secondary metabolite in Karo traditional oil, which make it a potent anti-inflammatory medicine [11-14].

Flavonoids, a group of naturally occurring benzo-γ-derivatives, are a compound found synthesized by vascular plants in response to microbial infection [2], binds with glucoside and flavonoid aglycone, which have abilities to show various biological properties such as antioxidant, antibacterial, anti-inflammatory, and antiviral effects [15]. To identify the flavonoid compound, the aglycones in plant extracts that have been hydrolyzed are examined. The extraction process is carried out with boiling ethanol to avoid oxidation. Karo traditional oil has flavonoids compound; they inhibit lipid peroxidation which occurs in injury therefore shown an increasing collagen fiber's strength by increasing the angiogenesis in the wound healing process and preventing cell damage [15]. Flavonoids in Karo traditional oil scavenge the reactive oxygen species and free radicals to prevent the oxidative stress [2,15].

The polyphenol phytochemistry screening is done to identify the secondary metabolite of Karo traditional oil using FeCl3 as the reactor. A positive result will be shown green, red, blue, purple, or black strong color. Karo traditional oil does not have any polyphenol secondary metabolite because there is no phenolic reaction's color appear in the middle test tube.

Saponin is a glucoside which dissolves in water and has bubble when shaking in the water with Liebermann-Burchard reactor. It has high toxicity and the ability to lysis the red blood cell. There are two kinds of saponin based on its structures, steroid frameworks saponin and triterpenoid framework saponin. Karo traditional oil does not have any saponin compound in it so it is not toxic and safe to use in wound care. Alpha-pinene was found to be the major component of Karo traditional oil, together with δ-carene, sabinene, limonene, and camphene. In this research, terpenoids are the dominant secondary metabolite in Karo traditional oil, which make it a potent anti-inflammatory medicine [11-14].

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Wound healing was accelerated by Karo traditional oil by suppressing the pro-inflammatory cytokines and promote anti-inflammatory cytokines, Karo traditional oil works as a potent anti-inflammation which regulates the inflammation phase of the wound healing process. Its effect on the proliferation and remodeling phase remains unknown. Therefore, it is necessary to do additional research to find the mechanism of action of this Karo traditional oil in the phase of proliferation and remodeling, which accelerates the re-epithelialization process.

CONCLUSION
Karo traditional oil contains terpine, alkali, and flavonoid compounds. Alpha-pinene was found to be the major component of Karo traditional oil, followed by delta-3-carene, octane, dodecan, camphor, undecane, isoocdecane, sabine, hexadecane, nonane, tridecan, cyclohexane, pentacosane, heptadecan, limonene, and camphene.

Alpha-pinene stimulates the inflammatory cell production, macrophage type-2 which is the key regulation of the wound healing process, but in the same time it suppresses the neutrophil migration, production of macrophage type-1 and pro-inflammatory cytokines, by blocking toll-like receptor-4 receptor which should be bound by DAMP and PAMP compound to stimulate the release of pro-inflammatory cytokines, this inhibits the NF-κβ, IkB kinase-β, and caspase-1 activation and suppress the MAPK pathway through decreasing of MAPK phosphorylation [ERK and [NK] in inflammatory cells and reduce the pro-inflammatory mediators such as interleukin-6, TNF-α, and cyclooxygenase-2 expression [11,13,14,16,17]. Alpha-pinene and other terpene compounds in Karo traditional oil play an important step in the inflammatory phase in wound healing [11].

C. nucifer oil is the solvent of Karo traditional oil; it also useful in wound healing treatment because of its short-chained and saturated fatty acids which prevent it from become mancid and suitable for the preservation of Karo traditional oil's bioactive compounds and for wound treatment [2].

Its effect on the proliferation and remodeling phase remains unknown [13]. Therefore, it is necessary to do additional research to find the mechanism of action of this Karo traditional oil in the phase of proliferation and remodeling, which accelerates the re-epithelialization process.

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CONFLICTS OF INTEREST
The authors whose names are listed in this article certify that they have no affiliations with or involvement in any organization or entity with any financial interesting the subject matter or materials discussed in this manuscript.

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