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Preliminary phytochemical evaluation of Kandikandilaan (*Stachytarpheta jamaicensis* Linn. Vahl) leaf extracts using gradient polarity solvents

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Abstract A preliminary phytochemical analysis of Kandikandilaan (*Stachytarpheta jamaicencis* Linn. Vahl) leaf extracts were conducted using solvents with different polarities: hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar). The findings indicated that the efficiency of extraction varied depending on polarity of the solvent used. The recovery of phytoconstituent was significantly impacted by the polarity of solvent. Ethanol gave the highest extraction yield among the solvents tested, indicating its ability of extracting a wide range of bioactive secondary metabolites. In particular, ethanol extracts tested strongly positive for tannins, alkaloids, phenolics, and amino acids, and moderately positive for saponins. Ethyl acetate, a semi-polar solvent, extracted only small amounts of tannins, phenolics, and terpenoids. In contrast, the non-polar hexane extract did not show detectable levels of any of the tested phytochemical constituents. These findings suggest that polar solvents like ethanol are significantly more effective at isolating hydrophilic bioactive compounds with potential therapeutic applications from Kandikandilaan.

Keywords: *Stachytarpheta jamaicensis,* Kandikandilaan, Phytochemical screening, Solvent polarity, Medicinal plants, Herbal extraction

Medicinal plants were rich in key phytochemical constituents includimg alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids. These constituents are crucial subjects of phytochemical observation because of their diverse biological properties (Harborne, 1998). Several plant-derived secondary metabolites particularly tannins, terpenoids, alkaloids and flavonoids have been showed to exhibit antimicrobial activity (Cowan, 1999). Because plant secondary metabolites differ in their chemical properties, particularly polarity and solubility, the selection of extraction solvent influences the types of compounds recovered. Polar solvents are generally offering optimal selectivity for extracting polar constituents, whereas non-polar solvents favor the extraction of less polar

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compounds. During fractionation, solvents of increasing polarity may be employed to facilitate the separation of compounds with different polarity characteristics (Abubakar and Haque, 2020; Lefebvre *et al.,* 2021).

Traditional medicine remains important source of healthcare in many parts of the World, particularly in developing countries where medicinal plants have long been use for health and disease management (World Health Organization (WHO), 2013). *Stachytarpheta jamaicensis* Linn. Vahl locally called Kandikandilaan which has a history of traditional medicinal use in the Philippines including the treatment of wounds and other ailments (Quisumbing, 1978). Despite this plant's significance in ethnomedicine, there are only few or limited scientific study on its phytochemical components and its potential wound healing activites.

The research finding aimed to evaluate the phytochemical components of *Stachytarpheta jamaicensis* Linn. Vahl leaves using extracts with hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar) representing solvents of different polarities and to characterize primary bioactive constituents present in the leaf extract.

Materials and methods

Collection, preparation and extraction of plant material

Newly gathered leaves of *Stachytarpheta jamaicensis* Linn. Vahl from Barangay Buneg Echague, Province of Isabela, Philippines on March 2025. The freshly gathered leaves were rinsed thoroughly under running water to eliminate surface dirt and foreign materials, followed by final rinsing with distilled water. Leaves were air-dried in a shaded area at room temperature (25–30 °C) for 7–10 days to preserve active constituents (Omran *et al.*, 2026). Once the leaves were completely dried, it was milled into a fine powder using grinder and kept in closely tight containers under dry, dark conditions until extraction.

About 300 grams of puverized leaves of Kandikandilan (*S. jamaicensis* Linn.Vahl) was soaked in 1000 ml 0f 95% ethyl alcohol for 48 hours following previously reported ethanol extraction for *S. jamaicensis* leaves (Idu *et al*., 2007) followed by filtration using whatman #1 filter paper to obtain ethanolic filtrates. Ethanol solvent was completely concentrated under reduced pressure using a rotary evaporator under low pressure below 45 °C to get viscous semi-solid masses. Then the extracts will be stored at 4-5 °C for further biological assays.

Fractionation of the bioactive constituents

The crude leaf extract of *S. jamaicensis* was subjected to fractionation using column chromatography to isolate and separate its bioactive constituents. Silica gel (60–120 mesh) was employed as the stationary phase. A gradient elution was used, beginning with non-polar solvent (n-hexane), using gradient elution system from low polarity using ethyl acetate (semi-polar) to high polarity using ethanol (polar).

The concentrated ethanolic extract was carefully loaded onto the top of the prepared silica gel column and eluted stepwise with solvent mixtures of increasing polarity. Eluted fractions were collected systematically in labeled vials or flasks. These fractions were then subjected to further phytochemical evaluation and characterization to detect the presence of major bioactive compounds and assess their potential biological activity.

Phytochemical Screening

The active fraction of the Kandikandilaan that are associated with wound healing based from related literatures was subjected to phytochemical screening for its different constituents known to have wound healing property according to the standard pharmacognosy and phytochemical analytical procedure and literatures as described by Harborne (1984) and Kokate *et al.* (1995).The solvent extract was screened qualitatively for the presence of major classes of phytochemicals using standard phytochemical tests as follows:

Tannin:Ferric chloride test

The ferric chloride test as described by Harborne (1984) relies on the reaction between ferric ions (Fe^{3+}) and polyphenolic groups that is present in tannins. Once 2 ml of the extract/chromatographic fractions and 2ml of the 5% ferric chloride solution are combined, the ferric ions cordinate with the phenolic hydroxyl group of tannins. This interaction yields a soluble dark green or blue-black complex. Apearance of dark green color indicates condensed tannins whereas hydrolyzable tannins produce a dark blue precipitate.

Saponins: Foam test

The foam test outlined by Kokate *et al.* (1995) evaluates saponins based on their amphiphilic molecular structure linked to 1 or more polar sugar sugar chains.Once 3 ml of the extract/chromatographic fractions was added with 2ml

of distilled water and shaken energetically for 15 minutes,the saponin molecules were alligned at the liquid-air interface.The orientation significantly reduces surface tension.trapping air particles into a cellular lattice that indicates formation of stable foam layer intact upon standing.

Alkaloid:Wagners test

For the detection of alkaloids according to Harborne (1984) methodology, 1 ml of the extract/chromatographic fractions was reacted with 1ml of Wagner reagent (Aqueous solution of iodine and potassium iodide). Reaction involves the nucleophilic addition of nitrogen present in the basic heterocyclic rings of alkaloids to the triodide ions(I_3^-). This results in the precipitation of reddish brown color.

Phenol:Ferric chloride test

The total phenolic compounds, as established by Kokate *et al.* (1995), was performed by adding 1 ml of the extract/chromatographic fractions with 1ml of the 5% ferric chloride. Hydroxide groups directly attached to aromatic rings bind with the trivalent iron ions to produce stable, colored complex salts. Apearance of dark green/reddish brown/blue/violet/purple signifies the presence of phenols.

Amino Acid:Xanthoproteic test

To test for the Amino acids using the protocol of of (Harborne,1984; Kokate *et.al*.,1995), 1 ml of extract/chromatographic fractions and was added with 1ml of concentrated nitric acid (white precipitate is formed), it was heated for 2-3minutes and cooled. Then 1ml of 20%NaOH is added, causing the nitro derivatives to convert into colored salts. The orange color produced indicates the presence of Aromatic Amino Acid

Terpenoid: Liebermann-burchard test

Terpenoid detection carried out in accordance with Harborne (1984), was performed by adding a 2 ml of extract/chromatographic fractions, 2 ml of chloroform and 2 ml of acetic acid and carefully added with 1 ml of concentrated sulfuric. Dehydration and oxidation of triterpenoids occur in the presence of strong acids, forming conjugated double bonds. Appearance of blue green color/reddish ring signifies the presence of terpenoids.

Observations during these testing procedures,following Harborne (1984) and Kokate *et al.* (1995) were recorded based on distinct visual color changes or precipitate formation, and results were reported qualitatively as either present (+) or absent (–)

Results

A qualitative phytochemical screening was conducted for Kandikandilaan leaf extracts using solvents of increasing polarity hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar). The result revealed marked differences in the presence and intensity of various bioactive constituents with the results based on color changes or precipitate formation as shown in Figure 1.

Among the tested solvents, ethanol (polar) extract demonstrated the broadest spectrum of phytochemicals. Strong positive reactions (+++) were observed for tannins (green color with ferric chloride), alkaloids (reddish-brown precipitate with Wagner's reagent), phenols (violet to dark green color with ferric chloride), and amino acids (orange color in Xanthoproteic test), as shown in Figure 1. Saponins showed a moderate foam formation (++), while terpenoids were absent in ethanol extracts. The ethyl acetate (semi-polar) extract exhibited low levels (+) of tannins, phenols, and terpenoids, based on mild color changes during their respective tests. However, it was not revealed the presence of any tested phytochemicals in hexane (non-polar) extract as presented in Table 1.

Table 1. Phytochemical Constituents Detected in Kandikandilaan (*Stachytarpheta jamaicensis* Linn. Vahl) Leaf Extracts Using Solvents 0f Varying Polarity

Phytochemicals	Non-polar extract (Hexane)	Semi- polar extract (Ethyl Acetate)	Polar extract (Ethanol)
Tannin	-	+	+++
Saponin	-	-	++
Alkaloid	-	-	+++
Phenol	-	+	+++
Amino Acid	-	-	+++
Terpenoid	-	+	-

Legend:
- absent
+ low
++ moderate
+++ high

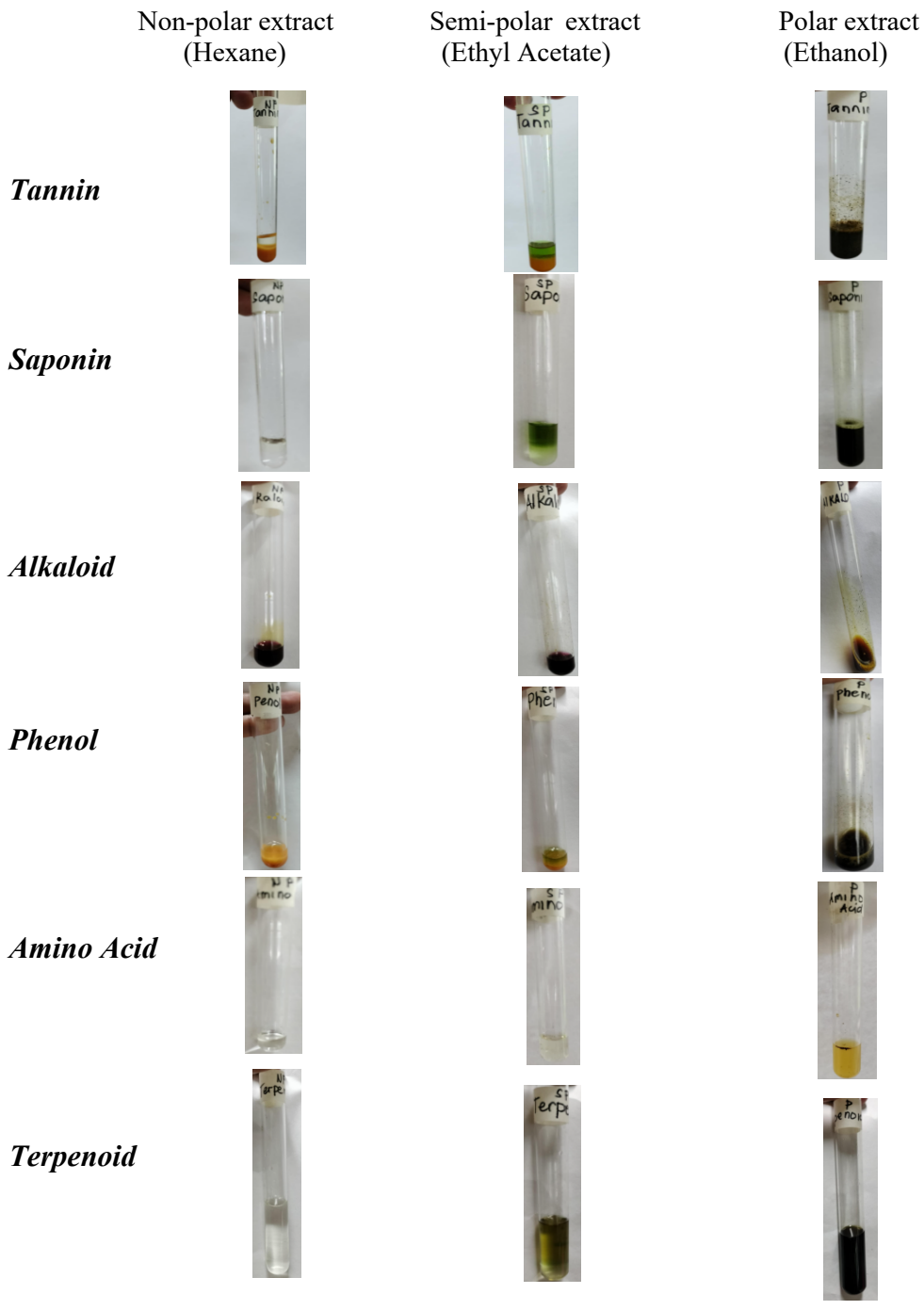


Figure 1. Visual results of phytochemical tests on Kandikadilaan leaf extracts using solvent of increasing polarity

Discussion

The study showed the phytochemical constituents of *Stachytarpheta jamaicensis* leaf extracts using solvents of varying polarity hexane, ethyl acetate, and ethanol and to relate these findings to the plant's ethnopharmacological uses, particularly in wound healing. The observed differences in phytochemical profiles among the extracts highlight the critical role of solvent polarity in the qualitative extraction of bioactive compounds.

Ethanol extract was the richest in antioxidant-related phytochemicals as confirmed with the findings of Azam *et al.* (2018) yielded the highest phytochemical diversity. This included high levels of tannins, alkaloids, phenols, and amino acids, as well as a moderate presence of saponins. These results are consistent with findings reported by Liew and Yong (2016), who identified tannins, alkaloids, and saponins in ethanol extracts. Previous reports that ethanol is effective in solubilizing polar constituents due to its capacity to disrupt plant cell walls and extract water-soluble phytochemicals (Harborne, 1998; Kokate *et al.,* 2001). These constituents are well documented for their wound healing, anti-inflammatory, and antimicrobial properties. Ethyl acetate extract as confirmed by Ruma and Zipagang (2015) also extracted phenolics and terpenoids, indicating its capacity to extract semi-polar compounds. Although less potent than ethanol in terms of diversity, ethyl acetate's detection of both polar and non-polar components suggests its potential for intermediate polarity compounds, including some secondary metabolites with therapeutic potential. The hexane extract did not exhibit any detectable phytochemical constituents, which can be attributed to the non-polar nature of hexane that limits its capacity to solubilize polar or hydrophilic compounds.

Overall, the findings are supported the traditional use of *Stachytarpheta jamaicensis* in the treatment of skin conditions. The presence of various phytochemicals especially in the ethanolic fraction gave a phytochemical rationale for its traditional application in wound care. In addition, these results are served as a foundational basis for further pharmacological studies and bioassay-guided fractionation to isolate and characterize the specific constituents responsible for therapeutic effects. Future studies may focus on quantitative phytochemical analysis, *in vivo* wound healing trials, and mechanistic evaluations to validate the biological activities of each compound class. The strong presence of key wound healing agents in the ethanol extract positions it as a promising candidate for the development of herbal formulations.

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Conflicts of interest

The authors declare no conflict of interest.

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