foam indicates the presence of saponins.

Detection of phytosterols - Detection of phytosterols had been carried using Salkowski's test. Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulfuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes. Libermann Buchard's test: extracts had been treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and allow cooling. Concentrated sulfuric acid was added. Formation of the brown ring at the junction indicates the presence of phytosterols.

Detection of phenols - Detection of phenols had been carried out using ferric chloride test. Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of tannins - Detection of tannins had been carried out using the gelatin test. The extract was added with 1% gelatin solution containing sodium chloride. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids - Detection of flavonoids had been carried out using the lead acetate test. Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence flavonoids.

Detection of diterpenes - Detection of diterpenes had been carried out using the copper acetate test. Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of Diterpenes.

Detection of terpenes - To 5 ml of the extract, add 2 ml of chloroform and 3 ml of Sulfuric acid (H_2SO_4) concentration. Formation of a reddish brown ring confirms the presence of terpenes.

Detection of coumarines - In a test tube 0.5 g of the moistened various extracts were taken. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Detection of steroids - A 0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulfuric acid. The color changed from violet to blue or green in some samples indicated the presence of steroids.

Detection of cardiac glycosides - A 5 ml of various solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

Detection of resins - Acetone-water Test: Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

Brine Shrimp Lethality Assay

Brine shrimp lethality assay had been carried out according to the principles and protocol described by Aguinaldo et al (2004) and Naidu (2013) with slight modification. Brine shrimp (*Artemia salina*) eggs hatched in artificial sea water prepared by dissolving 3.8 g rock salt in 100 ml distilled water. The container was covered with black cartolina, with aeration and illumination on the side of the chamber. After 48 hours if incubation at room temperature the larvae (active *nauplii*) were separated from the shells and are attracted to the brighter side of the hatching chamber. The *nauplii* were collected using micropipette. Ten active *nauplii* used for testing were placed in each vial containing 5 mL of artificial seawater solution. Tests have done along with control and different concentrations.

Preparation of Artificial Seawater

Artificial seawater was prepared by dissolving 3.8 g of rock salt per 100 mL distilled water.

Preparation of Different Solutions

In a solution A, 50 mg of Billy Goat weed extract was dissolved in 5 ml of methanol. For solution B, 0.5 mL of solution A was diluted in 10 ml methanol. After the solutions were prepared, the researchers pipette 100µL of solution B, 50 µL of solution A and 500µl of solution A into separate vials respectively and were labeled from 1 to 3 with 5 replicates each concentration. Then, a control vial containing 1 mL of methanol was made. The solution in each vial of concentration had undergone air drying at room temperature.

Hatching the Shrimp

Shallow rectangular dish (22cm x 32cm) was filled with artificial sea water; the rectangular dish served as the hatching dish of the brine shrimp. Then, a plastic divider was placed, punched with several 2mm holes in the dish which divide into two unequal compartments. The minute brown shrimp eggs were sprinkled into a larger compartment. The larger compartment was covered with black cartolina to keep away from light; a smaller compartment was leaved open. The smaller open compartment was then illuminated. The hatched brownish orange *nauplii* were pipette after 48 hours from the illuminated compartment of the dish.

Counting the nauplii

The *nauplii* were pipette and count microscopically in the stem of the pipette, held against a well-lighted background.

Preparation of Food for the Shrimp

A suspension of 3mg of dry yeast (red star) in 3ml artificial seawater was prepared for the food for the *nauplii.*

Concentration of sample vials 1, 2 and 3 and control vial

Each sample vials diluted to 5ml with artificial sea water makes a final concentration of 10,000 and1000 µg/ml respectively. With a 9 inch pipette, transfer ten nauplii into each sample vial labelled 1, 2 and 3 and control vial prepared. Add artificial sea water to each vial samples and control to make a total volume of 5ml. Add a drop of yeast suspension (3mg/5ml of sea water) as food in each vial. The vials were kept under illumination. The survivors were counted after 6 hours then after 24 hours. The record of the number of deaths after 24 hours was used in determination of the percent deaths for each dose level and for the control vials.

Lethality Concentration Determination

The surviving shrimps were counted using magnifying glass.

Survivors were counted after 24 hours and the percentage mortality at each vial and control has been determined using the equation:

$$\% \text{ mortality} = \frac{\text{no. of dead } nauplii}{\text{initial no. of live } nauplii} \text{ X100}$$

Statistical Tool

Probit analysis by Finney as discussed by Kim Vincent (2014) was used in determining the concentration at which lethality to brine shrimp represents 50% (LC50).

Results and discussion

Table 1 show the phytochemical constituents screening using the test tube method. It stated that Billy goat weed crude extract is positive for alkaloids, Saponins, glycosides, flavonoids, phenols, terpenes, coumarins, steroids, tannins, cardiac glycosides, resins. Phytosterols and Diterpenes are not present in Billy goat weed (*Ageratum conyzoides Linn*.). Different reagents were used in the determination of the phytochemical constituents in Billy goat weed (*Ageratum conyzoides Linn*.). They were Mayer's reagent distilled water, ferric chloride, lead acetate, sulfuric acid, acetic anhydride, copper acetate, sodium hydroxide, 1% gelatin solu-

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tion, and acetone. This implies that those phytochemical constituents with positive remarks are present in Billy goat weed (*Ageratum conyzoides Linn*.) while those with negative remarks are not present in Billy goat weed (*Ageratum conyzoides Linn*.). The result in table 1 is anchored to the study of (Naz and Bano, 2013) where the value of medicinal plants lies in phytochemical constituents that cause definite pharmacological action on the human body. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytochemical constituents like flavonoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpenes etc., are effective antimicrobial substances against a wide range of microorganisms.

These phytochemical constituents present in Billy goat weed (*Ageratum conyzoides Linn*.) crude extracts have beneficial effects to human. The phytochemical constituents present in Billy goat weed (*Ageratum conyzoides Linn*.) may be utilized for medicinal purposes. In the study conducted by Doughari (2012), alkaloids are known for their pharmacological applications as anesthetics and Central Nervous System (CNS) stimulants, the antibacterial properties, the anticancer agent, the anti-arrythmic, the pupil dilator, and the addictive stimulants; saponins has biological effects, inhibitory effects on inflammation, and possess antibacterial property according to Abioye et al. (2013); glycosides are neutral in reaction and can be readily hydrolyzed into its components with ferments or mineral acids. Glycosides are classified on the basis of type of sugar component, chemical nature of aglycone or pharmacological action according to Doughari (2012); flavonoids have long been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons free radicals, chelate metal catalysts , activate antioxidant enzymes , reduce alpha-tocopherol radicals, and inhibit oxidases according to Ukachukwu et al. (2013); phenols possess antimicrobial, antioxidants and cytotoxicity activities according to Sowunmi and Afolayan (2015); terpenes possess anti-microbial, anti-carcinogen, antioxidant, analgesic (painkiller), anti-inflammatory, muscle relaxer, and anti-depressant according to Vogeler (2010); coumarins possess anti-tumor activity according to Lacy and Kennedy (2004); steroids possess therapeutic applications and potent anti-malarial effects according to Adeyemi et al. (2014); tannins has anti-inflammation, anti-cancer, and ethno-pharmacological uses according to Sowunmi and Afolayan (2015); cardiac glycosides possess anti-cancer and anti-tumor according to Montaño et al. (2014); and lastly, the resins according to Amabye (2015) has anti-inflammatory properties.

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Table 1: Phytochemical Screening of Billy goat weed (Ageratum conyzoides Linn.)

Phytochemical Constituents	Reagents	Positive Results	Remarks
Alkaloids	Mayer's reagent	Yellow colored precipitate	+
Saponins	Distilled water	1 cm layer of foam	+
Glycosides	ferric chloride	Rose pink	+
Flavonoids	Lead acetate	Yellow Colored precipitate	+
Phytosterols	Sulfuric acid	Golden Yellow color	-
	Acetic anhydride	Brown ring	
Phenols	ferric chloride	Bluish black	+
Diterpenes	Copper acetate	Emerald green Color	-
Terpenes	Sulfuric acid	Reddish brown	+
Coumarins	Sodium hydroxide	Yellow Fluorescence	+
Steroids	Acetic anhydride	Violet to blue or green	+
Tannins	1% gelatin solution	White precipitate	+
Cardiac Glycosides	Sulfuric acid	Brown ring	+
Resins	acetone	Turbidity	+

(+) present; (-) absent

Table 2 shows the differences of death percentage of *nauplii* in different concentrations of ethanolic crude extract of Billy goat weed (*Ageratum conyzoides Linn*.). In the first concentration which is 10μg/ml, the death percentage of nauplii of 39.58%. The second concentration is 100 μg/ml; it has a death percentage of *nauplii* of 62.50%. Lastly, the third concentration is 1000 μg/ml; it has a death percentage of *nauplii* of 95.83%. Using the probit analysis, the LC_{50} of ethanolic crude extract of the Billy goat weed (*Ageratum conyzoides Linn*.) was computed to be 56.23 ppm (μg/ml). Cytotoxicity test performed on the ethanolic crude extract of Billy goat weed (*Ageratum conyzoides Linn*.) was the so-called brine shrimp lethality assay (BSLA). This implies the result in table 2 shows that the more concentrated the solution with the ethanolic crude extract of Billy goat weed (*Ageratum conyzoides Linn*.), the more potent it is.

Meanwhile, Peteros and Uy (2010) stated that the percentage mortality increased with an increase in concentration. It was supported by Del Socorro et al. (2014) that the maximum mortality observed at the highest treated-concentration whereas least mortality observed at the lowest treated-concentration. Based on the criteria set by Olowa and Nuñeza (2013), the extract is toxic or active if it has a LC_{50} that is lesser than 1000 µg/ml while it is nontoxic or inactive if the LC_{50} is greater than 1000 µg/ml. This goes to show that extract of Billy goat weed (*Ageratum conyzoides Linn*.) is indeed toxic. On the other hand another criterion was set on cytotoxicity using brine shrimp by Maridass (2008), which would require the LC_{50} value to be less than 250µg/ml in order to be considered toxic or active, so the cytotoxicity level of Billy goat weed (*Ageratum conyzoides Linn*.) based on Maridass criterion is active or toxic. It cannot be denied however that Billy goat weeds (*Ageratum conyzoides Linn*.) as a toxic material that can still be explored further. It should be noted that the computation of the LC_{50} was based on the percent mortality after 24 hours.

Table 1: Percent death and lethal concentration of Billy goat weed (Ageratum conyzoides Linn.) after 24 hours

Sample	Percent death after 24 hours			LC_{50} (µg/mL)
	10 µg/mL	100 µg/mL	1000 µg/mL	
Billy goat weed (*Ageratum conyzoides Linn.*) *extracts*	39.58%	62.50%	95.83%	56.23

Conclusion

It can be concluded that the crude extract contains alkaloids, saponins, glycosides, flavonoids, phenols, terpenes, coumarins, steroids, tannins, cardiac glycosides, resins. The toxicity level of Billy goat weed (*Ageratum conyzoides Linn*.) with LC_{50} of 56.23µg/ml is relatively active. In the light of the findings of the study, it is recommended that nutraceuticals and pharmacological analysis should likewise be performed. Phytochemical components identified should be isolated and further studied for structure elucidation. Other method such as Thin-layer Chromatography (TLC) should be explored as well in identifying phytochemical constituents. Different solvent should be explored in order to optimize the phytochemical screening and cytotoxic activity of Billy goat weed (*Ageratum conyzoides Linn*.).

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