

Research Article

Evaluation of Phytochemical Composition and Antibacterial Activity of *Indigofera hirsuta* Linn

Muhammad, M.U., Gayari, M.S., Idris, B., Garba, U and Bello, I

Department of Chemistry, Shehu Shagari College of Education, Sokoto

*Corresponding Author: buhariidris2017@gmail.com, doi.org/10.55639/607.1322

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ABSTRACT

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The phytochemical constituents of *Indigofera hirsuta* aerial part were assessed for their potential as sources of alternative medicines as well for industrial applications. Qualitative phytochemical screening of the extract revealed the presence of Flavonoids, Steroids, Alkaloids, Tannins, Saponins, Cardiac glycosides and Anthraquinones. Quantitative evaluation of the extract showed their concentrations to be Flavonoids (270.039 ± 0.013 mg/100g), Steroids (9.100 ± 0.007 mg/100g), Alkaloids (59.74 ± 0.014 mg/100g), Tannins (21.66 ± 0.03 mg/100g), Saponins (1.3 ± 0.0001 mg/100g) and Anthraquinones (0.205 ± 0.003 mg/100g). Our study indicated that the plant possessed different phytochemicals that determine its medicinal use. The crude methanolic extract was further tested at various concentrations to check its microbial activity against four bacterial pathogens. The results showed that the crude exhibited activity significantly at all concentrations against three organisms. (*Streptococcus pneumonia*, *Pseudomonas aeruginosa* and *Shigella dysenteriae*). Maximum activity was observed against *Streptococcus pneumoniae* (12 mm at 200 mg/ml, 11 mm at 100 mg/ml, 10 mm at 50 mg/ml and 9 mm at 25 mg/ml). While minimum level of microbial activity was observed against *Pseudomonas aeruginosa* (9 mm at 200 mg/ml, 8 mm at 100 mg/ml, 7 mm at 50 mg/ml and 6 mm at 25 mg/ml. However, *Proteus mirabilis* showed no activity). This result suggested the potential use of *Indigofera hirsuta* for the treatment of diseases caused by bacteria.

Corresponding author: Buhari Idris Email: buhariidris2017@gmail.com
Department of Chemistry, Shehu Shagari College of Education, Sokoto

INTRODUCTION

Medicinal plants contain a variety of metabolites such as alkaloids, saponins, flavonoids, tannins, anthraquinones, cardiac glycosides and steroids with antioxidant, antimalarial, anti-inflammatory and snake venom activities (Salem *et al.*, 2013). Medicinal plants play a vital role in disease prevention and their promotion and use fit all existing prevention strategies. However, during the design and implementation of these strategies, there needs to be a conscious effort to correctly identify, identify and locate medicinal plants. The medicinal value of plants lies in the fact that their phytochemical components have various physiological effects on the human body. Therefore, through phytochemical screening, a variety of important compounds can be detected, which can serve as the basis for modern drug treatment of various diseases (Sheikh *et al.*, 2013).

Indigofera hirsuta, hairy indigo or hairy indigo, is a flowering plant of the legume family. Native to almost all tropical regions of the world, South America, Africa, Southeast Asia and other places. It is harvested from the wild for local use as medicine and dye (Kumar *et al.*, 2010). *Indigofera hirsuta* has various medicinal properties. In Nigeria, leaf juice is used internally to treat liver disease, and a leaf decoction is used to treat jaw and ulcers, gravel, epilepsy or infantile convulsions, diabetes, leprosy, tuberculosis, infections, snake bites, malaria and eyelid inflammation. In Kenya, it is used as a chest medicine, while in Tanganyika, the whole plant is made topically to treat back pain. In India, it is used to treat brain disorders and to treat eye damage, malaria and eyelid inflammation, or to kill filarial worms in conjunctival tissue (Burkill, 1995).

It is well known that efficient extraction, determination and isolation of bioactive components from plant material is highly

dependent on the type of solvent used in the extraction process (Tiwari *et al.*, 2011). According to the literature obtained, the medicinal properties of this plant are due to the biologically active components. Therefore, this study aimed to evaluate the qualitative, and quantitative phytochemical constituents and Antibacterial activity of the indigo plant, which may have medicinal and industrial applications.

Materials and Methods

Sample Collection and Identification

The fresh aerial part of *Indigofera hirsuta* was obtained from Zaria town, Samaru Local Government of Kaduna State, Nigeria. The sample was identified and authenticated by the Herbarium Unit, Department of Botany, Faculty of Life Science, Ahmadu Bello University Zaria, with an identification voucher number V/N ABU0503.

Sample Preparation

Fresh aerial parts of *Indigofera hirsuta* were properly cleaned to remove dirt particles from the surface and washed carefully with distilled water, but not in excess, then shade dried for seven (7) days. Dried samples were pulverized and placed in air-tight plastic containers before extraction and further analysis.

Plant Extraction

Powdered plant material (1000 g) was weighed and soaked in five (5) litre gallons containing three (3) litres of methanol for seven (7) days, the extract was filtered using Whatman No 1 filter paper and the solvent was evaporated using a rotary evaporator. The resulting crude extract was dried and then weighed to determine the percentage yield. It was then subjected to bioassay analysis.

Preliminary Phytochemical Screening

Phytochemicals are bioactive constituents of medicinal plants which are not nutrients but are very useful to the plants. Some bioactive constituents of the methanolic extract of *Indigofera hirsuta* analyzed qualitatively are flavonoids, tannins, saponins, cardiac glycosides, steroids/terpenes, anthraquinones,

alkaloids, etc using standard procedures as follows:

Test for Alkaloids

Dragendorff's Reagent

Add three drops of Dragendorff's reagent to the 2 cm³ crude extract in the test tube. The formation of a reddish-brown precipitate indicates the presence of alkaloids (Trease and Evan, 2008).

Wagner's Reagent

Three (3) drops of Wagner's reagent were added to crude extract (2 cm³) in a test tube, formation of a dark brown precipitate reveals the presence of alkaloids (Trease and Evan, 2008)

Mayer's Reagent

Three (3) drops of Mayer's reagent were added to crude extract (2 cm³) in a test tube. An orange precipitate indicates the presence of alkaloids (Trease and Evan, 2008).

Test for Anthraquinones

Borntrager's Test: To the crude extract, 2 cm³ methanol and 2 cm³ of 10% ammonia solution were added. The formation of rose-pink colouration confirmed the presence of Anthraquinones (Trease and Evans 2008).

Test for Cardiac glycosides (Keller Kiliani)

A small quantity of crude extract (50 mg) was dissolved in 1 ml of glacial acetic acid containing some ferric chloride solution. The solution was transferred to a cleaned test tube followed by the addition of 2 cm³ of sulfuric acid. If brown ring forms at the interface, it indicates the presence of deoxysugars (Trease and Evans, 2008).

Test for Steroids/Terpenes

Liebermann-Buchard's Test: A small quantity of the crude extract was melted in chloroform (2 cm³). Then acetic anhydride (5 cm³) and concentrated sulfuric acid was added to the solution. The formation of green colouration shows the presence of steroids (Silva *et al.*, 1998).

Salkowski Test: The crude extract (0.5 g) was dissolved in chloroform (5 cm³) and

concentrated sulfuric acid (2 cm³) was then added to the mixture. The presence of steroids is confirmed by the formation of a red precipitate (Sofowora, 2008).

Test for Flavonoids

Ferric Chloride Test: Two (2 cm³) of 5% ferric chloride solution were mixed with crude extract (5 cm³). Green precipitate shows the presence of flavonoids (Trease and Evan 2008).

Test for Saponins

Dilute two (2) cm³ of an extract with 5 cm³ of distilled water and shake vigorously, then let stand for 15 minutes. Persistent foaming indicates the presence of saponins (Silva *et al.*, 2008).

Test for Tannins

Ferric Chloride Test: Distilled water (5 cm³) was added to a small quantity of the crude extract. The mixture was heated to boil for five (5) minutes, then added two drops of 5 % FeCl₃ were. The formation of greenish precipitate shows the presence of tannins (Trease and Evans, 2008).

Quantitative Analysis

Estimation of Alkaloids (Harborne Method)

An aliquot of 5 cm³ of the sample was added to acetic acid (20 cm³ of 10 %) in ethanol and covered the mixture with aluminium foil, and allowed to stand for 4 hours, then filtered. The filtrate was concentrated to a quarter of the original volume in a 500 °C water bath. Concentrated NH₄OH was then added to the filtrate, and the precipitate was collected through pre-weighed filter paper. This was followed by rinsing the precipitate with 2 cm³ of 1 % NH₄OH and filtered. The alkaloids precipitate was dried in an oven at 60 °C for 30 minutes, and the filter paper was reweighed (Harborne, 1998). the percentage of alkaloids content was calculated using equation (1):

$$\% \text{ Alkaloid} = \frac{\text{Final weight of sample}}{\text{Weight of sample}} \times 100 \quad (1)$$

Estimation of Flavonoids

Fifty (50) cm³ of 80 % aqueous methanol was added to 2.50 g of sample in a 250 cm³ beaker, covered with aluminium foil, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted with an equal volume of ethanol (3 times). The combined organic layers of the

sample were filtered through Whatman filter paper. The filtrate was then transferred to a crucible and evaporated to dryness in a 50 °C water bath. The contents were cooled in a desiccator and weighed till a constant weight was achieved. The percentage of flavonoids was calculated as follows (Ejikeme *et al.*, 2014):

$$\% \text{ Flavonoids} = \frac{\text{Weight of Flavonoids}}{\text{Weight of sample}} \times 100 \quad (2)$$

Estimation of Saponins

The extract was treated with ethanol (25 cm³, 20%) and then heated for 2 hours in a water bath with constant stirring at 55 °C. The extracted residue was added to 50 cm³ of 20 % ethanol. The combined extracts were reduced to 40 cm³ over a water bath at 90 °C. Then transferred to a 250 cm³ separatory funnel; 20 cm³ of ether was added and shaken vigorously. The aqueous layer was recovered, and the ether

layer was then discarded. 60 cm³ of n-butanol was added. The n-butanol extract was washed twice with 10 cm³ of 5 % aqueous sodium chloride. The remaining solution was heated in a 50 °C water bath. After evaporation, the samples were dried in an oven to constant weight. Saponins content was calculated as a percentage by equation (2) (Obadoni, *et al.*, 2001).

$$\% \text{ Saponins} = \frac{\text{weight of the saponins}}{\text{weight of the sample}} \times 100\% \quad (3)$$

Estimation of Steroids

An aliquot of 5 cm³ was taken from the powdered extract prepared in 50 cm³ of distilled water and shaken for an hour. Sulphuric acid (4M, 2 cm³) and iron (III) chloride (0.5% w/v, 2 cm³), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 cm³). Then, the mixture was heated in a water bath at 70 °C for 30 minutes with some shaking at intervals. Absorbance was measured at 780 nm relative to a reagent blank (Narendra *et al.*, 2013).

Estimation of Anthraquinones

The crude extract (5 cm³) was added to 10 cm³ of distilled water. The mixture was heated to boil for an hour and filtered and 5 cm³ Ethanol

(50 %) was added, the mixture was further filtered. The absorbance was measured at 450 nm (Khalid *et al.*, 2018).

Estimation of Tannins

The plant extract was added to a beaker containing 5 cm³ of distilled (and it was shaken for 1 hour, then filtered). 5 cm³ of the filtrate was measured in a beaker, and 2 cm³ of 0.1 M FeCl₃ in 0.1 M HCl and 0.008 M K₄Fe(CN)₆·3H₂O was added and the absorbance at 395 nm wavelength was measured within 10 minutes (Khalid *et al.*, 2018).

Thin Layer Chromatography

A 2 cm sharp pencil line near the bottom of the plate was drawn and a small drop of *Indigofera*

hirsuta crude methanolic extract was placed on its side by using a capillary. When the spots of the mixture were dry, the plates were immersed in a light solvent containing chloroform-ethyl acetate in a ratio of 7:2 in a TLC chromatography tank. It was ensured that the solvent level was below the line with the spot on it. The TLC chromatography vessel was then capped to ensure a saturated atmosphere of solvent vapours. As the solvent moves slowly upward, the different components of the extract are separated into different coloured spots (Li, 2014).

Antimicrobial Susceptibility Test

Bacterial cultures used in this study were obtained from Microbiology Department of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Bacterial cultures included in this study were *Streptococcus pneumoniae*, *Proteus mirabilis*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*. All

the cultures were grown in Muller-Hilton medium. The inoculum was used for antibacterial assay.

Four different concentrations (25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) were prepared from the methanolic crude extract of *Indigofera hirsuta* aerial part. The extract was used for antibacterial analysis using agar well incorporation methods (Nijs *et al.*, 2000). Plates of nutrients agar were prepared and allowed to solidify on Petri dishes and was then seeded with a test bacterium, four wells were made in each of the plate with a sterile 6.0 mm diameter cork borer. Each of the four wells was filled with a given concentrations of the extract mixed with plane sterile agar. The plates were then incubated at 37°C for 48 hours. The diameters of zones of inhibition were measured using a meter rule and the value for each organism were recorded.

RESULTS AND DISCUSSION

The Methanolic crude extract obtained from 1000 g of pulverized aerial part of *Indigofera hirsuta* by maceration method was (57.46 g),

representing (5.75 %w/w). The results of the qualitative, quantitative phytochemical analysis and TLC chromatography of the crude methanolic extract of *Indigofera hirsuta* were presented in Tables 1, 2 and 3.

Table 1: Results of Qualitative Phytochemical Constituents of *Indigofera hirsuta* Methanolic Crude Extract

Constituents	Test	Inferences
Alkaloids	Dragendorff's	++
	Mayer's	++
	Wagner's	++
Anthraquinones	Borntrager	+
Cardiac glycosides	Killer killiani	++
Flavonoids	Ferric chloride	+++
Tannins	Ferric chloride	++
Saponins	Frothing	++
Steroids/terpenes	Lieberman Buchard	++
	Salkowski	++

Key; Highly present: +++, moderately present: ++ and lightly present: +

The results of qualitative phytochemical analysis of the crude extract obtained from *Indigofera hirsuta* aerial part revealed the presence of flavonoids, Tannins, alkaloids, saponins, cardiac glycosides, steroids/terpenes and Anthraquinones as presented in Table 1. Plant extracts contain good amounts of alkaloids. Similar observations were also recorded in *Indigofera tinctoria* plants, as reported by Chandrasekaran (2018). Alkaloids are one of the main reported medicinal compounds present in plant parts. They are reported to have analgesic, antispasmodic and bactericidal properties. In humans, most alkaloids affect the nervous system, especially the action of chemical transmitters such as acetylcholine and epinephrine. Some alkaloids are also used as preservatives due to their antibiotic activity (Arpita, 2017). Cardiac glycosides were also found to be moderately present in crude extracts, an observation consistent with that reported by Efuntoye *et al.* (2014) for *Indigofera arrecta*. Cardiac glycosides have been reported to have specific potent effects on the myocardium and, in very small amounts, can produce beneficial stimuli for heart disease (Nagy, 2017). The presence of saponins in crude plant extracts may be a reason for common traditional use in muscle relaxation and wound treatment in northern Nigeria (Okwu *et al.*, 2001). Qualitative testing of this plant extract showed the significant presence of saponin content. Similar observations were made with *Indigofera trita* and *Indigofera tinctoria* plant extracts, as reported by Ramamurthy *et al.* (2017) and Chandrasekharan (2018) respectively.

Steroid triterpenes/terpenes were observed in the plant extract, which is consistent with other species of the same family such as *Indigofera trita* (Ramamurthy *et al.* 2017). Steroids have been reported by Mahmoud *et al.*, (2021) with numerous biological functions, including antiviral, antispasmodic, antihyperglycemic, anti-inflammatory, and immunomodulatory properties. Steroids have been reported to have antivenom activity in mice (Aliba *et al.*, 2018). Crude extracts of the plant show appreciable amounts of flavonoids. This observation is corroborated by the literature for *Indigofera trita* and *Indigofera tinctoria* species as reported by Chandrasekaran (2018) and Ramamurthy *et al.*, (2017), respectively. Flavonoids have been reported to have various activities, such as antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant and antidiabetic effects (Kalsi *et al.*, 2013).

Significant tannin content was also observed in the extract, which compared well with that of the papers reported by Efuntoye *et al.* (2014) and Ramamurthy *et al.* (2017) for *Indigofera arrecta* and *Indigofera trita*, respectively. Crude extracts of *Indigofera hirsuta* also revealed the presence of anthraquinones. These photochemical compounds have antiviral, antifungal, antibacterial, laxative, insecticidal and antioxidant properties, making them suitable for the treatment of various diseases, such as the treatment of constipation, cancer, etc. (Nida, 2019). Therefore, the medicinal properties of the plant claimed by traditional healers may be due to the presence of these physicochemical compositions in the plant.

Table 2: Results of Quantitative Analysis of Crude Methanolic Extract of *Indigofera hirsuta*

Constituents	Quantity
Alkaloids (mg/100g)	59.74 ± 0.014
Flavonoids (mg/100g)	270.39± 0.013
Saponins (mg/100g)	1.31± 0.0001
Anthraquinones (mg/100g)	0.205± 0.003
Steroids (mg/100g)	9.10± 0.007
Tannins (mg/100g)	21.66±0.05
Cardiac glycoside	ND

Values represent mean ± standard deviation of duplicate determination; ND = Not Determined.

Quantitative analysis of the crude methanolic extract showed that the level of flavonoids in the indigo plant was higher than that of the other phytochemicals investigated and was recorded as (270.39 ± 0.013 mg/100g). This was evidenced by the qualitative results (Table 1). Flavonoids are known to have antioxidant effects and have been shown to inhibit tumorigenesis (Kim *et al.* 1994). A reduction in coronary heart disease has been reported to be associated with flavonoid intake (Hertog *et al.*, 1993). In addition to the antioxidant properties of flavonoids, it has other biological functions, including protection against platelet aggregation, microorganisms, heparin, viruses, tumours, ulcers, free radicals, inflammation, and allergies (Barakat *et al.*, 1993). The number of tannins in the extract was 21.66 ± 0.05 mg/100g. Tannins play a very important role as raw materials for sustainable green industries. Mainly used in leather, feed, fishery, beverage and other industries. They also find application as potential medicinal agents, antioxidants, and metal chelators; and cater as inhibitors of harmful pro-oxidation processes. Several other important properties, such as the preservative, anticancer, and anti-inflammatory properties of tannins, have been demonstrated in humans, making them suitable candidates for the pharmaceutical and nutraceutical industries (Akhilash *et al.*, 2019). The Alkaloid content in the extract was (59.74 ± 0.014 mg/100g) as shown in Table 2. Alkaloids are composed of most compounds containing naturally basic nitrogen atoms and are found mainly in plants, but may also be produced by bacteria, fungi and animals. In this study, appreciable quantities of alkaloids were obtained from crude extracts of buttercup, which could serve as an inexpensive and stable way to provide this secondary metabolite in industries that need them. According to the literature, alkaloids have a wide range of

pharmacological activities, including antimalarial (eg quinine), anticancer (e.g homologous brine) (Kittakoop *et al.*, 2014), antibacterial (eg celandine) (Cushnie *et al.*, 2014) and antihyperglycemic activity (eg piperine). Alkaloids are also used for their importance in traditional medicine. Other alkaloids are psychoactive (eg, psilocin) and stimulants (eg, cocaine, caffeine, and nicotine), and have been used recreationally (Qiu *et al.*, 2014).

The content of Anthraquinones was 0.205 ± 0.003 mg/100g which is in agreement with the results of the qualitative analysis as shown in Table 1. Anthraquinones are an important class of natural and synthetic compounds with a wide range of applications such as relief of constipation, arthritis, multiple sclerosis and cancer (Malik and Muller, 2016). Steroids were also detected in the extract and found to be 9.10 to 0.007 mg/100 g. Steroids play a key role in many diseases, including malignancies such as prostate cancer, where the production of steroids inside and outside the tumour promotes the aggressiveness of cancer cells (Lubik *et al.*, 2016).

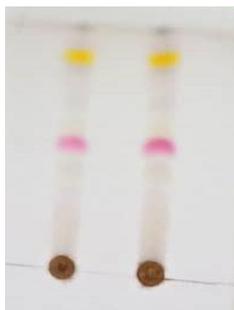
The number of saponins detected was (1.31 ± 0.0001 mg/100 g). Saponins have many health benefits, with studies showing benefits in blood cholesterol levels, cancer, bone health and immune system stimulation. Saponins bind to bile salts and cholesterol in the gut. Bile salts and cholesterol from small micelles aid their absorption. Saponins also lower blood cholesterol by preventing their absorption. Plants also produce saponins to fight parasitic infections, and when ingested by humans, saponins also appear to help our immune system and protect against viruses and bacteria. The non-sugar portion of saponins also has direct antioxidant activity, which may lead to other benefits such as the reduced risk of cancer and heart disease (Zetner, 2011).

Table 3: Thin Layer Chromatography on Crude Extract of *Indigofera hirsuta*

Position of spots	Solvent front	Spots distance from origin	Colour of spots	Rf value
1	5.40	2.30	Light brown	0.43
2	5.40	2.70	Pink	0.50
3	5.40	4.20	Light brown	0.78
4	5.40	4.30	Light pink	0.80
5	5.40	4.60	Yellow	0.85
6	5.40	4.80	Light blue	0.89
7	5.40	5.30	Green	0.98

The thin layer chromatography of *Indigofera hirsuta* methanolic crude extract conducted with chloroform-ethyl acetate in a ratio (7:2) solvent system, sprayed with 10 % H₂SO₄ and heated in an oven at 110 °C showed the appearance of seven spots as shown above on TLC plate (Fig. 1). It clearly shows three major spots and five minor spots with light colourations.

The results of this study strongly support the preliminary phytochemical screening and quantitative analysis of the crude extract. The appearance of the number of spots from the TLC chromatogram indicates that the methanolic crude extract of *Indigofera hirsuta* is a mixture of different components.

**Figure 1:** TLC plate of the crude methanolic extract of *Indigofera hirsuta*

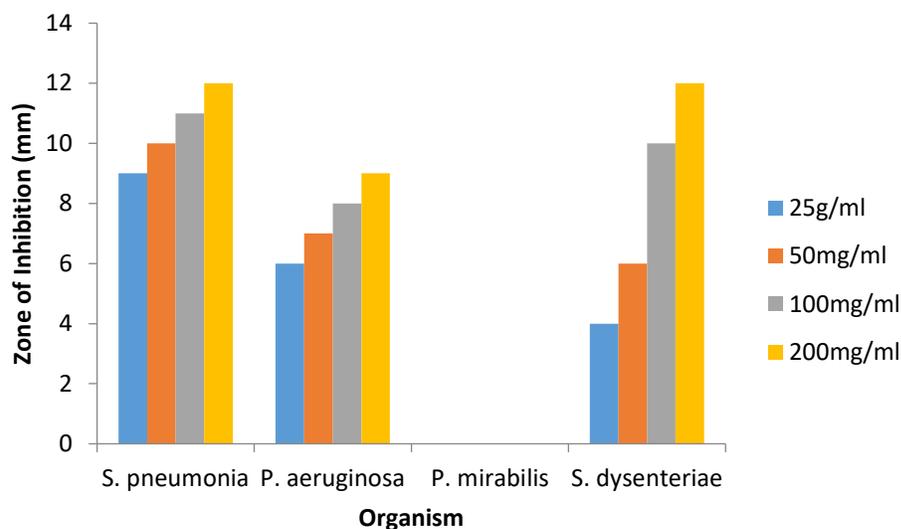


Figure 2: Zone of Inhibitions (mm) of *Indigofera hirsuta* Crude Methanolic Extract

Antimicrobial activity was carried out against *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Shigella dysenteriae* at various concentrations (25, 50, 100 and 200 mg/ml) of the crude methanolic extract. The results showed that the crude extract had significant antibacterial activity against *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Shigella dysenteriae* at all concentrations. The highest zone of inhibition is against *Streptococcus pneumoniae* (12 mm at 200 mg/ml, 11 mm at 100 mg/ml, 10 mm at 50 mg/ml, and 9 mm at 25 mg/ml). *Shigella dysenteriae* shows moderate activity against the tested bacteria with zone inhibitions of (12 mm at 200 mg/ml, 10 mm at 100 mg/ml, 6 mm at 50 mg/ml and 4 mm at 25 mg/ml) while the microbial strain against *Pseudomonas aeruginosa* has the lowest bioactivity (9 mm at 200 mg/ml, 8 mm at 100 mg/ml, 7 mm at 50 mg/ml, and 6 mm at 25 mg/ml). *Proteus mirabilis* showed no activity at all concentrations as shown in (Figure 2). The activity observed from the plant extract against the tested organisms may be attributed to the secondary metabolite present. The same observations for *E. coli*, *Salmonella*

typhi and *Vibrio cholera* were reported by Kumar *et al.* (2017).

CONCLUSION

This study showed that the aerial parts of *Indigofera hirsuta* contain significant amounts of flavonoids and alkaloids, as well as several of other phytochemicals tested in crude extracts. The use of this plant in the treatment of different ailments and medicinal claims of the plant could be attributed to the identified bioactive compounds present. Our study confirmed that the crude extract has effective resistance to the growth and reproduction of clinical pathogens. Advances in pharmacological activities are being studied, which will justify the claims of traditional healers for the medicinal properties of the plant.

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Conflict of interest

The authors declare that there is no conflict of interest.

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