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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF *MIRABILIS VISCOSA*

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ABSTRACT

Plants have long been utilized for both culinary and therapeutic purposes. Commercially, phytochemical study of the plant is quite important, and there is a lot of interest in it for potential medicines. The aim of the current study was to explore the important bioactive components of leaf extracts from *Mirabilis viscosa* in both qualitative and quantitative analyses using polar solvents such as water, methanol, ethanol, acetone, and ethyl acetate. The non-polar solvents are hexane and chloroform. Standard techniques were employed to determine the phytochemical components' qualitative and quantitative analysis. A qualitative study of *M. viscosa* revealed the presence of secondary metabolites, including alkaloids, protein and amino acids, carbohydrates, reducing sugar, and saponin. Phytosterols, Glycosides, Tannins, Glycosides, Phenols, Coumarins, Triterpenoids. Through quantitative analysis, the highest concentration of total flavonoids (40.91± 0.01 mg/g QE) in ethyl acetate was found. Quercetin was used as a standard, and total alkaloids (26.11± 0.005 mg AE/g) in ethanol were found to be equivalent to atropine sulphate. Total phenol (2.07± 0.29 mg GAE/g) in methanol was found to be equivalent to gallic acid. Additional study on the plant will be helpful in identifying and analysing its secondary metabolites, which will be very helpful in treating a variety of illnesses and in the pharmaceutical industry in the production of new medications. Further bioactive components from the plant will be isolated, and these might eventually be applied to medicine.

Keywords: Qualitative and Quantitative, Secondary metabolites and *Mirabilis viscosa*.

Introduction

India contains a large diversity of medicinal plants, many of which are still understudied. Nature has gifted us with therapeutic herbs that enable us to live long, healthy lives free from illness. Plant-based natural products have long been a staple in the creation of curative and prophylactic remedies (Nostro *et al*., 2000; Abad *et al*., 2012; Alves & Rosa, 2007). The World Health Organization states that traditional medicine, which includes herbal medicine, has been and is still utilized in some form in practically every nation on earth. For primary healthcare, more than 70% of the world's population uses medicinal herbs (WHO, 2008). Since the beginning of time, people have utilized plants as medical treatments. The use of herbs as an alternative form of treatment to preserve health and enhance quality of life is greatly increasing (Nostro *et al*., 2000). The health of individuals and societies greatly depends on medicinal plants. Certain phytochemical components found in plants have therapeutic significance and influence on human physiology (Rahmatullah *et al*., 2009). Plant has therapeutic properties that are attributed to phytochemicals. These substances, which are not food, but people are shielded from a number of illnesses (Lamaaeshwari & Ananti, 2012). Phytochemicals are main and secondary components found naturally in medicinal plants such as leaves, grains, vegetables, roots and among other places. Secondary compounds include flavonoids, alkaloids, sterols, terpenoids, saponin, tannins, and other substances not found in primary compounds (Savitramma *et al*., 2011). Chlorophyll, proteins, and carbohydrates are examples of primary compounds. On the other hand, flavonoids,

alkaloids, sterols, terpenoids, saponin, tannins, and other secondary compounds are examples of secondary constituents (Motaleb, 2011).The chemical substances found in plants called phytochemicals are a vital source of both food and medicine. Numerous biological activities, including antibacterial, antifungal, and antioxidant properties, are known to exist in them. Typically, secondary metabolites including alkaloids, flavonoids, tannins, and other phenolic compounds are the significant bioactive substances found in plants (Edeoga *et al*., 2005). In accordance with Harborne, phytochemicals are separated and extracted using various methods, and then their biological activity is assessed (Harborne *et al*., 2013). *Mirabilis viscosa* is a herbaceous species that belongs to the Nyctaginaceae family. It grows freely in Mysuru and its surroundings. A native of Colombia, Ecuador, Peru, and Mexico. The common name is "Viscid Umbrella-Wort," originating from the fruit's unique characteristics. Herbs, either short-lived perennials or annuals, erect, branching plants. Stems became almost thicker and more brittle at nodes. leaves that are acute to obtuse at the tip, The three anthers were dorsifixed, upright, and longitudinally dehiscing stamens in terminal inflorescences. Monocarpellary, Unilocular and one ovule. Fruit is persistent involucres, 3 mm long, 5-ribbed, verrucose, and greyish brown in appearance (Kumara *et al*., 2012). The primary focus of this work is the preliminary screening of qualitative separation & quantitative determination of primary and secondary metabolites from the leaf extracts of Methanol, ethanol, hexane, chloroform, water, ethyl acetate, and acetone of *Mirabilis viscosa.*

Materials and Methods

A healthy *Mirabilis viscosa* plant leaf part was gathered from the Mysuru district, Mysuru, Karnataka, India. The plant was authorized by Botanical Survey of India, Coimbatore, Tamil Nadu.

Preparation of plant extracts

To remove the dust particles that had adhered, these leaves were cleaned with distilled water. They were allowed to dry in the shade. The dehydrated leaves were pulverized, weighed, and kept in sanitized containers.

Soxhlet extraction

A Soxhlet apparatus was used to extract 5g of dried plant powder using a hot continuous per site method for 4–5 hours. The solvent used was 150ml of polar, non-polar, and dipolar solvents such as ethanol, methanol, acetone, ether, and chloroform. In order to obtain a Soxhlet crude extract, water was evaporated on a hot water bath until it was completely dry. The solvent was then concentrated using a rotary flash evaporator.

Qualitative Phytochemical analysis

Standard techniques with slight modification were used to analyse the plant phytochemistry (Harborne, 1998).

Detection of carbohydrate

Molisch's Test

To the 1ml of plant extract, add a few drops of Molisch reagent, add Concentrated sulphuric acid was carefully to the sides of the test tube. Carbohydrates are indicated by a brown ring at the interface.

Detection of reducing sugar

Fehling's Test

Add equal parts of Fehling solutions A and B to the 1ml of extract, then shake it. Precipitate that is brick red suggests the presence of reducing sugars.

Benedict's Test

Add 2 ml (10 drops) of Benedict's reagent ($CuSO_4$) to 1 ml of plant extract. A boiling water bath is used to heat the solution for three to five minutes. precipitate formation or a change in colour in the test tube solution. When the colour turns green or brick red when it boils, sugar is present in the solution.

Test for proteins and Amino acids

Biuret test:

Add 2 ml of the plant extract, 5 drops of 1% hydrated copper sulphate, and 2 ml of 40% sodium hydroxide. Shake the test tube vigorously. After five minutes, the colour changes. Proteins are shown by a purple colouring.

Ninhydrin test:

In test tube with 1 mL of the extract. Add a few drops of the 2% ninhydrin reagent to the test tube and mix well. After five minutes in a boiling water bath, remove the test tube and let it cool to room temperature. The presence of α-amino acids is indicated by the emergence of purple to blue colour.

Millon's Test:

Add 1 ml of extract to a test tube. Mix thoroughly after adding two to three drops of Millon's reagent. retained for roughly two minutes in the water bath. A reddish-pink precipitate and a white precipitate appear in Millon's test, indicating a successful outcome. This suggests that tyrosine or a protein containing tyrosine is present.

Test for saponin

Foam test:

Add a few millilitre (ml) of extract to the test tube. Give it a good shake to generate foam. Hold on and give it for five-minutes. If after five minutes the foam is still there. The test verifies that the extract contains saponin.

Froth test

Add 3ml of the extract into a sterile test tube. Add 10 ml of distilled water to that. After the test-tube was sealed, it was forcefully shaken for roughly five minutes. After letting the test tube stand for thirty minutes, the honeycomb froth was looked for. If the honeycomb foam that remains after 30 minutes suggests that saponins are present,

Test for alkaloids

Dragendorff 's test

Prepare a test tube with 2 ml of extract. Add 1 mL of the Dragendorff reagent to that. Give it a good shake. The precipitate turned orange-red, signifying the existence of alkaloids.

Mayer's test

Fill a test tube one ml with plant extract. Add a few drops of Mayer's reagent. Shake well to ensure proper mixing. the precipitate forms in the test tube. A favourable outcome will be indicated by the production of cream-colored (yellowish or white) precipitates in the test tube. Alkaloids are so found in the sample of plants provided.

Wagner's test:

Take 1 mL of plant extract. Then 1mL of Wagner's test reagent was added. Shake gently to mix properly. The appearance of a whitish, cream, or reddish-brown precipitate signifies the existence of alkaloids.

Test for Flavonoid:

Alkaline test

Place a few drops of diluted NaOH (2%) solution into 1 ml of plant extract. There was a bright yellow colour in the test tube. In the event that the solution turns colourless, add a few drops of diluted HCL. which would suggest the presence of flavonoids

Ferric chloride test:

Add 1 mL of plant extract in a test tube and add a tiny amount of neutral ferric chloride solution and mixed thoroughly. The development of a greenish-black hue suggests the presence of phenolic chemicals.

Shinoda test:

Add 3 ml of plant extract, a tiny bit of magnesium hydroxide, and a few drops of dilute hydrochloric acid. the development of a red colour, which suggests the presence of flavonoids.

Test for Coumarins:

NaOH test:

Add 10% NaOH to 3 ml of plant extract, and then give it a good shake. A solution with a yellow colour suggests the presence of coumarins.

Test for Triterpenoids

Salkowski test:

Add 1 ml of chloroform to 3 ml of plant extract, stir, and then add 1 mL of conc. H_2SO_4 was gradually added to the test tube sidewalls. the development of a strong reddish-brown tint, which is suggestive of the existence of phenolic group or terpenoids.

Test for phytosterols

Acetic anhydride test:

Add 2 drops of Conc. H_2SO_4 to 1 ml of plant extract after 10 drops of acetic anhydride. and shake it then watch for colour changes. A colour that is initially red or violet, then blue, and finally turns green, indicates the presence of sterols.

Test for Cardiac glycosides

Killer killiani test:

In a test tube, add 1 ml of plant extract and 3 ml of glacial acetic acid. Shake the test tube. Add a few drops of 5% ferric sulphate solution to that combination, and then put one or two drops of pure sulfuric acid inside the tube walls. At the intersection of two liquid layers, a reddish-brown layer that is beginning to take on bluish shades of green develops.

Test for Phenolic Compound

Ferric Chloride Test

Fill a test tube with 1 ml of plant extract. Subsequently, a tiny quantity of neutral ferric chloride solution is added and thoroughly mixed. when red, blue, purple, or blackish green coloration forms Phenols are present.

Lead Acetate test

Fill a test tube one ml of plant extract. One ml of 1% lead acetate solution was then added and mixed thoroughly, and check for any precipitate. Precipitate formation suggests that phenols are present.

Test for tannins

Gelatin Test

Place 1 ml of the extract into a dry, clean test tube. Add the 10% sodium chloride solution after that add1% gelatin solution. After thoroughly mixing, look for any white precipitate. precipitate formation in the event of tannin presence.

NaOH Test

Put 0.5 ml of a 20% sulphuric acid solution into a clean, dried test tube with 1 ml of extract. Add a few drops of the aqueous sodium hydroxide solution and stir carefully. It becomes blue, a sign that tannins are present.

Test for glycosides:

Bontrager's test

Add 2 mL of extract and 2 ml of diluted sulfuric acid into a test tube. After 5 minutes of boiling, strain the mixture. The test tubes were then filled with an equivalent volume of chloroform. Ammonia in modest amounts is added to separate the organic layer. The ammonia layer's pinkish red colour suggests the presence of glycosides.

Legel's test

Pour 1 ml of the extract into a test tube, then add a drop of 20% sodium hydroxide solution and a few drops of nitroprusside and shake, The production of a rich crimson colour indicates the presence of glycosides.

Quantitative phytochemical analysis

Total alkaloid contents (TAC)

10 ml of chloroform was used to wash the mixture three times. Adding distilled water to make up to 1 ml of the leaf extract (20 µl). Then pH was subsequently adjusted to neutral using 0.1N NaOH. After that, the extract was combined with 5 mL of bromocresol green solution and 5 mL of phosphate buffer. Afterwards, 1 ml of chloroform was added to the mixture and stirred strongly until 10 ml of chloroform was present. Using a spectrophotometer against a reagent blank, the absorbance of the solution was measured at 470 nm. The correlation equation y = 0.0027x, which was generated with different concentrations of atropine sulphate (10-100 µg), was used to compute the total alkaloid content. The amount of alkaloid content is given as the same as that of atropine sulphate (Ajanal *et al*., 2012).

Total flavonoids content (TFC)

The extract of the leaf (20 µl stock) was combined with 4 ml of distilled water, then 0.3 ml of 5% $NaNO_2$ and 0.3 ml of 10% $AlCl_3$ were added. For five minutes, the mixture was left to stand at room temperature. After the incubation period, 2 ml of 1 mM NaOH was added, and distilled water was used to bring the total volume to 10 ml. Using a UV-Vis spectrophotometer and a reagent blank, the absorbance was measured at 510 nm. The correlation equation y = 0.0011x, which was generated with various concentrations of quercetin (10-100 µg), was used to determine the total flavonoid content. The amount of flavonoid content is represented as the quercetin equivalent (Kim *et al*., 2003).

Total phenols content (TPC)

A 20 µl stock of leaf extract was collected, and 200 µl of distilled water were added to the volume. One millilitre of 10% FC reagent was added to the sample, and it was then incubated for five minutes at 37°C. A millilitre of 6% Na_2CO_3 was then added, and the mixture was incubated for 30 minutes at 37°C. The UV-Vis spectrophotometer was used to detect the absorbance at 750 nm after the incubation period in comparison to the reagent blank. Using a standard graph with different concentrations of gallic acid (10-100 µg), the correlation equation y = 0.923x was used to compute the total phenolic content. Gallic acid equivalent was used to represent phenolic content (Gao *et al*., 2000).

Results and Discussion

Qualitative phytochemical analysis.

Plants are useful for medicine because they contain certain chemicals that affect human physiology in specific ways. Numerous phytochemicals have been shown to have a variety of functions, which may aid in the prevention of long-term illnesses. A preliminary phytochemical investigation of *Mirabilis viscosa* leaf extracts revealed the existence of bioactive substances. The analysis revealed the presence of primary and secondary metabolites, including carbohydrates, reducing sugars, protein and amino acids, saponin, alkaloids, flavonoids, phenols, coumarins, triterpenoids, phytosterols, cardiac glycosides, tannin, and glycosides. The results of the qualitative phytochemical analysis are listed in Table 1.

Tabel 1 : The Qualitative phytochemical analysis of leaf extracts of *M. viscosa*

SL No	Phytochemicals	Test	Leaf Extracts						
			Water	Ethanol	Methanol	Hexane	Chloroform	Acetone	Ethylacetate
1	Carbohydrates	Molisch's test	-	+	+	+	+	+	+
2	Reducing Sugar	Fehling's test	+	-	-	-	-	-	-
		Benedict's test	+	+	-	-	-	-	-
3	Proteins and Amino acids	Biuret test	+	-	-	-	-	-	-
		Ninhydrin test	+	+	-	-	-	-	-
		Millon's test	+	+	+	-	-	+	+
4	Saponin	Foam test	+	-	-	-	-	-	-
		Froth test	+	-	-	-	-	-	-
5	Alkaloids	Dragendroff' test	+	+	+	-	+	+	+
		Mayer's test	-	+	+	-	-	+	+
		Wagner's test	+	+	+	+	+	+	+
6	Flavonoids	Alkaline test	+	+	-	-	-	-	-
		Ferric chloride test	-	+	+	-	-	+	-
		Shinoda test	+	+	-	-	-	-	+
7	Phenols	Ferric chloride test	-	+	+	-	-	+	-
		Lead acetate test	+	+	+	-	-	-	-
8	Coumarins	NaoH test	-	-	-	-	-	-	-
9	Triterpenoids	Salkowski test	+	-	-	-	-	+	-
10	Phytosterols	Acetic anhydride test	-	+	+	+	+	+	-
11	Cardiac glycosides	Killer Killiani test	-	+	+	+	+	+	+
12	Tannins	Gelatine test	-	+	+	-	-	+	-
		NaoH	-	-	-	-	-	-	-
13	Glycosides	Bontrager's test	+	+	+	-	-	+	-
		Legel' S test	+	+	+	-	-	+	-

(+: Presence of Phytochemicals, -: Absence of Phytochemicals)

The results obtained indicate that the leaf extracts of *M. viscosa* exhibited the highest concentration of phytochemical components, including flavonoids, phenols, and alkaloids, across all extracts. The extracts of hexane, chloroform, and ethyl acetate lacked flavonoids and phenols. Every extract contained carbohydrates, with the exception of the aqueous extract. Except for the ethanol and water extracts, reducing sugar was not present in any of the extracts. All extracts contained proteins and amino acids, with the exception of hexane and chloroform. There was only saponin in the aqueous extract. It was observed that acetone and aqueous extracts contained triterpenoids. Both the aqueous and ethyl acetate extracts lacked phytosterols. All of the extracts contained cardiac glycosides, with the exception of the aqueous extract. Ethanol, methanol, and acetone all included tannin. Glycosides were not present in the hexane, chloroform and ethyl acetate extracts. In every extract, coumarins was absolutely missing. The two fuels with the most phytochemical potential were methanol and ethanol.

Quantitative phytochemical analysis

The examination of secondary metabolites is essential for the extraction, purification, separation, crystallization, and identification of different phytocompounds. The main phyto compounds in the leaf extracts, such as total flavonoids, total phenols, and total alkaloids, were then quantified using a conventional process.

Total alkaloid contents (TAC)

In Graph 1, the atropine sulphate standard calibration curve displayed a linear regression of $Y = 0.003x - 0.0173$ $R^2 = 0.9763$.The unit of measurement for the total alkaloids was mg AE/g, or atropine sulphate equivalent. in Table 2. Ethanol extract had the highest total alkaloid content (26.11 ± 0.005), followed by aqueous extract (10.93 ± 0.001), acetone (8.33 ± 0.001), ethyl acetate (4.44 ± 0.001), chloroform (3.70 ± 0.001), hexane (2.78 ± 0.002), and methanol (2.41 ± 0.002). The triplicate outcomes were expressed as mean ± standard deviation. Comparing extracts made with different solvents, ethanol revealed the largest overall alkaloid concentration, while methanol showed the lowest concentration of alkaloid.

Table 2 : Total Alkaloid content

Extracts	Total Alkaloid- (mg AE/g)
Water	10.93 ± 0.001
Ethyl acetate	4.44 ± 0.001
Ethanol	26.11 ± 0.005
Acetone	8.33 ± 0.001
Methanol	2.41 ± 0.002
Hexane	2.78 ± 0.002
Chloroform	3.70 ± 0.001

Values are mean ± SD (n=2); AE= atropine sulphate;

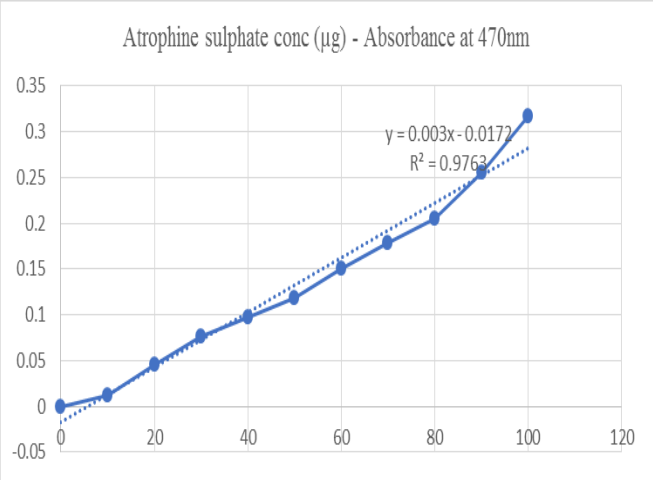


Fig. 1 : Standard curve of Atrophine sulphate for Alkaloids determination

Total flavonoids contents (TFC)

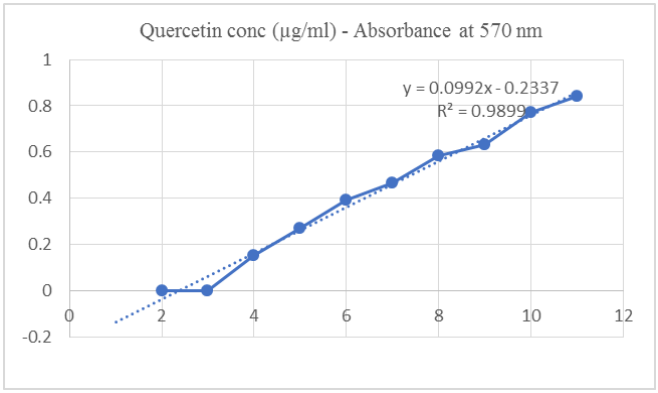
The linear regression of Y = 0.0922x - 0.2337 R^2 = 0.9899 was displayed in Graph 2, for the standard calibration curve of quercetin. In Table 3, the total flavonoid content was represented as mg/g QE, or quercetin equivalent. The collected data were presented as the mean ± SD of three duplicates. As indicated in Table 3, the flavonoid concentration of the ethyl acetate extract was greater at 40.91 ± 0.01, followed by that of the aqueous and methanol (27.27 ± 0.03 & 27.27 ± 0.01), acetone (22.73 ± 0.01), and ethanol (20.45 ± 0.01). When compared to other solvent extracts, ethyl acetate extract showed the highest total flavonoid level, while ethanol showed the lowest total flavonoid content.

Table 3: Total flavonoid content

Extracts	Total Flavonoid-mg/g QE
Water	27.27 ± 0.03
Ethyl acetate	40.91 ± 0.01
Ethanol	20.45 ± 0.01
Acetone	22.73 ± 0.01
Methanol	27.27 ± 0.01

Values are mean ± SD (n=2); QE= quercetin equivalent;

Fig. 2 : Standard curve of quercetin for flavonoids determination



Total phenols content (TPC)

A linear regression of Y = 0.0914 + 0.0007 R^2= 0.9963 was displayed in Graph 3 for the standard calibration curve of gallic acid. The total content of phenolic was expressed as Gallic acid equivalent (mg GAE /g). Gallic acid equivalent (mg GAE /g) was used to express the overall phenolic content. The results were presented as mean ± SD of triplicates, as shown in Table 4.With a total phenolic content of 2.07 ± 0.29, the methanol extract was the most prevalent, followed by the acetone extract (1.80 ± 0.29), ethyl acetate (1.53 ± 0.29), ethanol (1.34 ± 0.23), and water (1.26 ± 0.17).

Table 4 : Total phenolic content

Extracts	Total Phenol -mg GAE /g
Water	1.26 ± 0.17
Methanol	2.07 ± 0.29
Ethanol	1.34 ± 0.23
Acetone	1.80 ± 0.29
Ethyl acetate	1.53 ± 0.29

Values are mean ± SD (n=2); GAE= gallic acid equivalent;

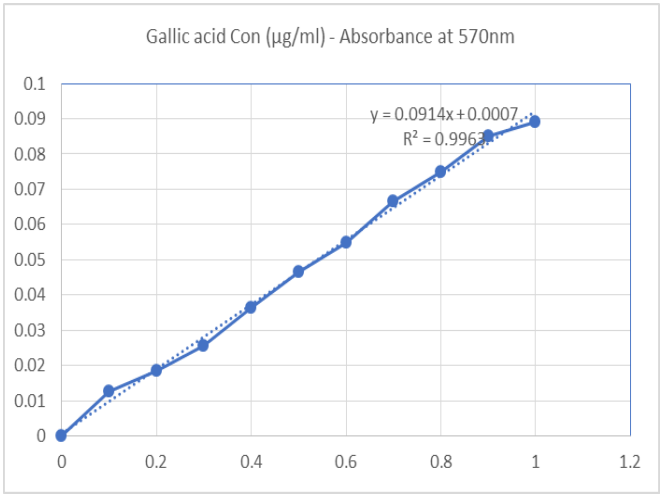


Fig. 3 : Standard curve of gallic acid for phenolics determination

Standard procedures were used to quantitatively evaluate the total amount of phytochemicals present in the *M. viscosa* leaf extract across all solvents. The current study demonstrates a notable variance in the contents, including phenols, alkaloids, and flavonoids. The highest total flavonoid concentration was observed, succeeded by the highest total alkaloid and total phenolic values. In contrast to the outcomes listed above. These variations are caused by a variety of environmental elements, including rainfall, altitude and climate (Kokate *et al*., 2004). It is essential to use different solvents depending on polarity in order to extract desired phytochemical components from plant materials (Pradeep *et al*., 2016). Based on their biological functions and structures, phytochemical substances are non-dietary plant derivatives that are classified as secondary metabolites. These are the principal substances that possess a wide range of qualities, such as antioxidant and antibacterial capabilities. They function as widespread pathogens (Dushimemaria *et al*., 2012). It is well recognized that flavonoids possess antioxidant properties because they eliminate free radicals, which are extremely unstable chemicals that cause harm to bodily cells and are linked to a number of illnesses, including cancer, inflammation, heart disease, and early aging. The antipyretic, analgesic, and spasmolytic (spasm suppressing) effects are attributed to flavonoids (Olaleye, 2007). Alkaloids are essential to medicine and make up the majority of expensive medications. Among living things, they have significant physiological effects. Alkaloids constitute a broad class of secondary metabolites that have been shown to block DNA topping enzyme, which has an antibacterial effect. Alkaloids provided protection against long-term illnesses, and it has been previously noted that alkaloids found in bitter leaf can lessen headaches induced by hypertension (Ayitey *et al*., 1997; Bonjean *et al*., 1998). The control of plant development, growth, and disease resistance is dependent on the increased phenol content. A diet high in plant-based polyphenols can protect against the development of osteoporosis, diabetes, cardiovascular disease, cancer, and neurological illnesses. Phenols and phenolic compounds continue to be the standard by which other bactericides are assessed due to their extensive use in disinfections (Akinyeye *et al*., 2014). When developing novel medications to address a range of illnesses, pharmaceutical companies as well as research organizations equally find commercial value in the phytochemical analysis of medicinal plants. Consequently, we think that the important phytochemical traits found in this study were the source of *M. viscosa* wide range of pharmacological activity.

Conclusion

The results of this study show that, due to a possible source of phytochemicals discovered in this plant, the qualitative and quantitative analysis of *M. viscosa* leaf extracts reveal the presence of numerous secondary metabolites with medicinal properties that can be used in traditional medicine to treat various kinds of infections. These metabolites are also responsible for the plant's antimicrobial and antioxidant activities. Future research is necessary to isolate, identify, characterize, and clarify the structure of biologically active substances. There is a possibility that this study will yield a novel compound that will lead to the development of new medications for the treatment of various ailments.

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