COMPARATIVE ANALYSIS OF QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL EVALUATION OF SELECTED LEAVES OF MEDICINAL PLANTS

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Abstract

When it comes to treating numerous ailments, India's traditional medicine has fared better than synthetic contemporary medicine, has less side effects, and is less expensive. The current study's goal was to compare the qualitative and quantitative analyses of the phytochemical components of leaves from Tinospora cordifolia (Wild) Hook.f. and Murraya koenigii (L.) Spreng., both of which were obtained from Mahabaleshwar. The cold extraction method was used to extract ethanol from the powdered, shade-dried leaves. These ethanolic extracts were examined using suggested laboratory procedures for phytochemical analysis. The data were statistically analyzed using the one-way analysis of variance (ANOVA) and Tukey's multiple comparisons at probability value (p 0.05). Alkaloids, flavonoids, tannins, terpenoids, steroids, saponins, phenols, and glycosides were discovered by phytochemical analysis. The highest phenol and alkaloid concentrations were found in Murraya koenigii (1960.7166.88 and 19.420.26).

INTRODUCTION

Worldwide, medicinal plants have long been recognized as a rich source of therapeutic substances for the treatment and prevention of diseases and maladies. (1). Up to 80% of the world's population, according to the World Health Organization, still relies on conventional therapies like herbs for their main medical care. (2). Ayurvedic, Siddha, and Unani medicine are just a few of the traditional medical systems that have been adopted in India. The Ayurvedic medical systems largely use plants and herbal remedies to treat a variety of ailments. (4). Many ailments may now be treated successfully with synthetic drugs, yet some individuals still choose to employ traditional folk remedies because they have fewer adverse effects. (5).

The medicinal plants have produced more than 13,000 secondary metabolites that have been isolated. In plants, secondary metabolites carry out specialized tasks or act as defense chemicals. These secondary metabolites have therapeutic benefits, such as antioxidant and anti-diabetic action. (6,7). There have been reports of the anti-diabetic activity of alkaloids, phenolics, terpenoids, flavonoids, saponins, xanthones, polysaccharides, and other substances. (8).

Because they contain chemical components that have a particular physiological effect on the human body, plants have medicinal significance. Essential bioactive chemicals found in plants include alkaloids, flavonoids, tannins, and phenolic compounds. (9). Tinospora cordifolia (Wild) Hook.f. and Murraya koenigii (L.) Spreng. are two plants that are frequently found in Mahabaleshwar and are used in traditional folk medicine to cure a wide range of illnesses. These plants have a lot of bioactive substances in their leaves, such as polyphenols, alkaloids, and flavonoids, which have a range of bioactive qualities,

such as antioxidant, anticancer, antibacterial, antidiabetic, and hepatoprotective activities. (10-13).

Phytochemicals are naturally occurring substances found in various plant sections that can protect the liver by offering nutrients or medical benefits. The place where the various medicinal plants are grown, the temperature there, as well as the manner and timing of collection, all have a significant impact on the phytochemical compositions of those plants. (14). The purpose of the current study was to compare the qualitative and quantitative analysis of the phytochemical components of the leaves of the two medicinal plants previously described**.**

MATERIALS AND METHODS

Collection of plant materials

The selected fresh leaves of two medicinal plants i.e. *Murraya koenigii* (L.) Spreng., *Tinospora cordifolia* (Wild) Hook.f were collected from Mahabaleshwar hill from September to October 2020. These plants were botanically authenticated.

Preparation of plant materials

To avoid the direct loss of phytoconstituents from sunlight, the freshly harvested leaves were air-dried methodically at room temperature for three weeks after being repeatedly cleaned with tap water to remove soil and dust particles. The pulverizer was used to grind the shade-dried plant leaves, which were then sieved through up to 80 meshes. After being homogenized into a fine powder, it was stored separately in airtight containers at room temperature (31°C) for subsequent investigation.

Preparation of plant extracts

extraction method. Each plant's leaves were ground into a total of 50 g, which was weighed separately, and put into 500 ml culture flasks. It was then given 150 mL of 1:3 pure ethanol (1: 100% ethanol) and thoroughly mixed. Each bottle's lid has parafilm covering it. Using a shaker set at 150 revolutions per minute for 15 minutes every morning and evening, the solution was stored for five days with periodic shaking. These were subsequently run through Whatman No. 1 filter paper. A portion of the filtered content was concentrated using a rotatory evaporator (Buchi), and a second portion was stored for later use at 4°C in the refrigerator. The analysis was done for three replicates of each medicinal plant leaf.

Qualitative analysis of phytochemicals (15-20)

To identify the presence of various phytochemicals such as alkaloids, flavonoids, tannins, steroids, glycosides, phenols, terpenoids, saponins, coumarins, anthraquinones, and quinines, the preliminary phytochemical screening of the ethanol extracts of each medicinal plant's leaves powder was conducted using recommended laboratory procedures.

Phytochemical screening for flavonoids (alkaline reagent test)

A few drops of 20% NaOH were combined with each 2 mL of the filtered sample. It was seen that a bright yellow tint was forming. The yellow color vanished after the addition of a few drops of hydrochloric acid that had been diluted by 70%. The appearance of flavonoids is indicated by the creation and disappearance of the yellow color.

Phytochemical screening for phenols (ferric chloride test)

Each 2 mL of filtered sample was mixed with 2 mL of 5% aqueous FeCl3.The formation of the blue color points out the occurrence of phenols.

Phytochemical screening for tannins (ferric chloride test)

Each 2 mL of filtered sample was added with 10% of alcoholic FeCl3. The formation of the black/brownish blue directs the occurrence of tannins.

Phytochemical screening for alkaloids (Dragendroff‟s test)

Each 2 mL of filtered sample was dissolved individually in dilute hydrochloric acid and filtered. The filtrate was treated with Dragendroff‟s reagent (solution of potassium bismuth iodide). The formation of a red precipitate indicates the presence of alkaloids.

Phytochemical screening for terpenoids (chloroform test)

Each 2 mL of filtered sample was added with 0.5 mL chloroform with 0.5 mL of acetic anhydride and a few drops of concentrated sulfuric acid. The formation of reddish-brown precipitate directs the presence of terpenoids.

Phytochemical screening for anthraquinones

Each 2 mL of filtered sample was added with potassium hydroxide. The blood red colour shows the presence of anthraquinones.

Phytochemical screening for saponin (foam test/frothing test)

Each 2 mL of filtered sample was added with 4 mL of distilled water. It will be mixed well and shaken vigorously. If foam will be produced continues for ten minutes, it designates the presence of saponins.

Phytochemical screening for quinones

Each 1 mL of filtered sample was added with 1 mL of sodium hydroxide. The formation of blue, green, or red colors shows the presence of quinones.

Phytochemical screening for coumarins

Each 1 mL of 1% filtered sample was added with 3-4 drops of 1% KOH in absolute ethanol. The formation of yellow color directs the occurrence of coumarins.

Phytochemical screening for glycosides (Keller-Kiliani test)

Each 2 mL of filtered sample was added with 0.5 mL glacial acetic acid, three drops of 1% aqueous FeCl3 solution, and 0.5 mL H2SO4 concentrated. A brown ring formed between the layers, which showed the entity of cardiac steroidal glycosides.

Phytochemical screening for steroids

It was carried out by Salkowski's test. About 2 mL of sample was mixed with 2 mL of chloroform. Then, 2 mL of concentrated H2SO4 was added to it. If steroids are present, the chloroform layer will appear red, and the acid layer will show greenish-yellow fluorescence.

Quantitative analysis of phytochemicals

Quantitative analysis for total phenolic content (Folin-Ciocalteu colorimetric method)

About 20 µL of each filter was added to the test tube using a micropipette. 1.58 µL was added to each above test tube. 100 µL of Folin-Ciocalteu reagent was added to each test tube. They were mixed well using a magnetic stirrer and allowed for eight minutes after stirring. 300 µL of a sodium carbonate solution was added to each stirred solution. They were heated in a water bath at 40°C for 30 minutes. They were permitted to cool. They were again stirred well. The Absorption of each sample was measured using a spectrophotometer at 765 nm wavelengths.

Quantitative analysis for total flavonoid content (aluminum colorimetric method)

4.5 mL of distilled water were added to each 0.25 mL filtered sample. A 0.3 mL solution of 5% NaNO2 was added, and 5 minutes were permitted. After mixing, 0.3 mL of 10% AlCl3 was incubated for 5 minutes. The entire volume was made to 10 mL with distilled water and thoroughly mixed before 2 mL of 1N NaOH was added. Using a spectrophotometer, the absorbance of each sample was determined at 510 nm. With the aforementioned chemicals and distilled water in place of sample, a blank was created. With the help of absorbance and concentration, a curve chart was created for each solution. For each sample, the three replicates were created. The amount of flavonoids in each gram of dry matter was determined as mg catechin equivalent (mg CAE).

Statistical data analysis

The results were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons at probability value ($p \leq 0.05$) using the SAS statistical program (version 9.1.3). In each analysis, three replicates were maintained for each sample.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals

Using a qualitative study of the leaves from two chosen medicinal plants, the presence or absence of phytochemicals was determined. Table I presents the findings. According to the study, both plants contain saponins. Saponins have a wide range of abilities, including the capacity to bind cholesterol as well as precipitate and coagulate red blood cells. Additionally, it has hemolytic activity and the generation of foam in aqueous solutions. Saponins have historically been used as molluscicides and detergents. Saponins are used in industry as foaming and surface-active agents, but they also have positive health effects against a number of ailments. (25).

Table I. Preliminary phytochemical screening of ethanolic extracts of selected plant leaves

Phytochemicals	*M. koenigii*	*T. cordifolia*
Tannin (black colour)	+	+
Saponins (foam)	+	+
Flavonoid (yellow color)	-	+
Alkaloid (red precipitate)	+	+
Quinone (green or red color)	-	-
Anthraquinones (blood red color)	-	-
Glycoside (brown ring)	+	+

Terpenoids (reddish-brown precipitate)	+	-
Steroids (greenish yellow fluorescence)	+	+
Phenol (blue color)	+	+
Coumarins (yellow color)	+	+

(+): Presence; (-):- Absence

Plant steroids are used in the production of food, herbal medicines, and cosmetics and are important for their cardiotonic effects. In order to activate the bone marrow and encourage growth, steroids are employed. It encourages lean body mass and helps older men prevent bone loss. (26). Steroids were identified as a result of this study in all four plants. The anti-hypoglycemic effect of terpenoids from herbal plant origin has been the subject of numerous studies. Terpenoids are present in Murraya koenigii. Additionally, flavonoids exhibit a range of vital functions, such as antihyperglycemic action. (27). Flavonoids are detected in *T. cordifolia*, the study claims.

The findings demonstrate that both of the chosen plants contain alkaloids. Thus, it can be said that alkaloids are one of the healing agents found in medicinal plants, and numerous natural bio-resources that have been explored may prove to be important in naturopathy and possess qualities that might be further investigated. (28). Numerous bioactive compounds found in herbal plants have the potential to be effective medicinal agents. Glycosides have a high potential for healing diabetes mellitus and many other illnesses, according to earlier study. (29). The findings of this investigation indicate that the glycosides are present in all four plants. In medicinal plants, a number of naturally occurring phenolic compounds have anti-inflammatory, antioxidant, antibacterial, and neuroprotective activities. Results reveal that all of the chosen plants contain phenols.

The findings show that all of the chosen plants lack quinone and anthraquinones. All of the chosen plants have coumarins. Several of the biochemical and pharmacological characteristics of coumarins have potential therapeutic use in the treatment of diabetes and its consequences. (30). Tannins may have analgesic, anti-diabetic, and anti-inflammatory actions, among other things. Tannins may one day prove to be an effective kidney-relieving drug. The findings indicate that tannins are present in all of the chosen plants. These phytochemicals significantly affect the hypoglycemic response. (31). Consequently, they aid in lowering diabetes. Both antibacterial and antihyperglycemic activities are present in them.

An exciting method for creating bioactive products and pharmaceuticals that may one day serve as useful therapeutic aids is the use of natural chemical compounds from plants as antibacterial and antifungal agents.(32).

Quantitative analysis of phytochemicals

Table II lists the results of a quantitative phytochemical examination of the leaves of various medicinal plants. A large and diverse family of chemicals known as phenolic compounds is made up of several secondary aromatic metabolites that are present in plants. Among other biological

functions, it has been claimed to have antioxidant, anti-diabetic, and antibacterial actions. (33). There is a wide spectrum of pharmacological effects for phenolic substances. The primary sources of phenol's antioxidant activity are its redox properties, hydrogen donors, and singlet oxygen quenchers. (34). In drug-herb interactions, gallic acid has also been demonstrated to have a synergistic role that increases therapeutic effectiveness while minimizing negative effects. The findings of this investigation demonstrate that *M. koenigii* and *T. cordifolia* were considerably different in terms of total phenolic content. Curry leaf ethanolic and aqueous extracts have moderately significant antioxidant activity at all concentrations, and this antioxidant activity rises with sample concentration.

Table II. Quantitative analysis of phytochemicals of selected plant leaves

Plant species	Phenol (mgGAE/g)	Flavonoid (mgCAE/g)
M.koenigii	1960.71 ± 66.88	15.42 ± 3.50
T. cordifolia	325.61 ± 23.84	15.03 ± 1.42

Plants respond to microbial infection by producing hydroxylated phenolic chemicals called flavonoids, which have been shown to have antibacterial effects in vitro against a variety of pathogens. The antioxidative properties of flavonoids are attributed to a number of mechanisms, including the scavenging of free radicals, the chelation of metal ions like iron and copper, and the inhibition of free radical-producing enzymes. (35). Catechins reduce blood sugar while also controlling insulin release because of their anti-hyperglycemic properties. Additionally, catechins have antiviral qualities. The findings of this study indicate that *M. koenigii* had a higher total flavonoid content than *T. cordifolia*.

CONCLUSION

Medicinal plants and phytochemicals have much importance in the present scenario in developing countries where resources are limited. Regular uptake of herbal medicines containing these phytochemicals can benefit many health problems. The results of preliminary phytochemical screening using ethanolic extracts of *M. koenigii* , and *T. cordifolia*, leaves are presented in this work. Leaves of *M. koenigii* are rich in critical specific phytochemicals and higher amounts of total phenolic and flavonoid contents than other plants. Therefore, *M. koenigii* can be used as multi-functional medicinal herbs in the traditional system of medicine and to prepare ready-to-use functional products and nutraceuticals.

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