Evaluation of Different Components of *Cannabis Sativa* and *Murraya Koenigii* for Antioxidant and Antibacterial Activity

Matharu M.[1], Kaur R.[1]

[1]ADI Intellect, 400 Tradecenter Dr, Woburn, MA 01801, United States.

Corresponding author details

Name: Kaur R.

Address: ADI Intellect, 400 Tradecenter Dr, Woburn, MA 01801, United States.

Email: nishu.rooproy16@gmail.com

Abstract

Purpose: Herbal plants are rich in tannins, alkaloids, flavonoids, and phenolic compounds. Antioxidant supplements or meals containing antioxidants may help the body's defense system. Medicinal plants also inhibit the growth of different microorganisms like bacteria, fungi, viruses, etc. This study is exploring similar components in commonly available local plants, such as *Cannabis sativa* and *Murraya koenigii*.

Methods: An organic solvent was used to make a medical plant leaf extract (Methanol). The ability to reduce ferric ions was measured by the ferric reducing antioxidant power assay (FRAP), and the agar-well diffusion method was used to test the antibacterial activity of methanolic plant extracts. Plant extracts' antibacterial effectiveness is determined by the type and amount of phenolic content in the tissue.

Results: Analysis of *Murraya koenigii* leaves showed high levels of antioxidants such as phenolics and flavonoids but an absence of tannins, saponins, and volatile oils. *Cannabis sativa* samples showed positive levels of alkaloids, flavonoids, terpenes, steroids, and resins. The leaf extract was subjected to a phytochemical examination to evaluate the presence of phenol, flavonoids, tannins, alkaloids, saponins, and terpenoids. Plant extracts' antibacterial effectiveness is determined by the type and amount of phenolic content in the tissue.

Conclusion: *Murraya koenigii* and *Cannabis sativa* extracts have shown high antioxidant activity. Both plants' extracts have antibacterial action against a variety of microorganisms. The findings highlight the importance of scientific research to support ayurveda in the development of future drugs.

Keywords: *Murraya koenigii, Cannabis sativa,* antioxidant, antibacterial, microorganisms.

Introduction

Evaluation of new therapeutic products is a time-consuming and difficult process, but medicinal plants may be a feasible option because they have been studied by different ethnic groups since ancient times. For nearly 85% of the world's population, medicinal plants are regarded as the primary source of healthcare.[1]

More than 40% of the synthetic medications on the market are produced from natural compounds derived from plants and microbes.[2] Herbal plants are rich in tannins, alkaloids, flavonoids, and phenolic compounds. These can be used to treat a wide range of degenerative conditions.[3,4]

The human body is extensively exposed to external sources of free radicals in today's world. As a result, the body's antioxidative defense system may not be sufficient to fully avoid oxidative damage. Antioxidant supplements or meals containing antioxidants may help the body's defense system and contribute to the reduction or neutralization of oxidative damage in this way.[5]

Medicinal plants also inhibit the growth of different microorganisms like bacteria, fungi, viruses, etc. The antibacterial properties of plants like *P. erinaceus* and B. salicina are well established.[6,7] The most famous use of medicinal plants for the cure of infectious diseases is the discovery of artemisinin from Artemisia annua for the treatment of malaria.[8] Plant extracts from *Dioscorea batatas, Glycyrrhiza radix, Mollugo cerviana, and Polygonum multiflorum Thunb* have also shown activity against coronaviruses.[9,10] As a result, a number of investigators in Asia and Africa have investigated the activities of plant extracts, fractions, and pure chemicals present in the extracts on a variety of microorganisms related to bacterial and fungal illnesses.[11-13] This study is also exploring similar components in commonly available local plants, such as *Cannabis sativa* and *Murraya koenigii*, in order to meet rising healthcare demands in a cost-effective manner.

Methods

Extract preparation

Herbal plant leaves (*Murraya koenigii* and *Cannabis sativa*) were collected in sterile containers. Medicinal plant leaves were cleaned, measured, and washed many times with water to eliminate dirt before being air-dried in a shed at room temperature (26˚C) for two weeks. Then, using an electronic grinder or mortar and pestle, it was crushed to a homogenous powder and stored in sterile containers for later use. An organic solvent was used to make a medical plant leaf extract (Methanol). For 2-3 days, the dry plant powder was soaked in methanol with intermittent shaking. It was filtered via Whatman filter paper at the end of the extraction procedure. This methanolic filtrate was concentrated at 40˚C on a rotary evaporator under reduced pressure and then stored at 4˚C. This methanolic filtrate was concentrated at 400˚C on a rotary evaporator under reduced pressure and then stored at 40˚C for later use. To get a known concentration of methanol extract, the filtrate was reconstituted in a known amount of DMSO.

Phytochemical analysis

Cannabis sativa

The leaf extract was subjected to a phytochemical examination to evaluate the presence of flavonoids, tannins, phenols, saponins, terpenes, steroids, resins, and volatile oils. Handpicked 500g of leaves of C. sativa were separated from stems and twigs and then crushed using an electronic grinder. 5g of dried powdered leaves were soaked in 200 ml of petroleum ether for 24 hours at 25˚C to make a stock solution of C. sativa. After evaporating the solvent at room temperature (26˚C), 25 ml of acetone was used to dissolve the resin, yielding a solution with a concentration of 12 mg/ml.

Murraya koenigii

Murraya koenigii L. leaves were completely washed 2-3 times with running tap water and once with sterile water, air dried for 6-7 days in the shade, then mechanically pulverized using a wooden mortar and pestle. The pulverized plant material was stored in airtight containers to keep it dry. The leaf extract was subjected to a phytochemical examination to evaluate the presence of phenol, flavonoids, tannins, alkaloids, saponins, and terpenoids.

Antioxidant activity

DPPH assay

The sample's radical scavenging potential was measured by measuring the drop in absorbance caused by DPPH at 517nm, which represents the creation of its reduced form, 2,2-diphenyl-1-picrylhydrazine (DPPH), which is yellow in color. The purple-colored methanolic solution shows high absorption at 517nm due to the presence of odd electrons in the sample.[14]

FRAP assay

The ability to reduce ferric ions was measured by the ferric reducing antioxidant power assay (FRAP). The flavonoids and phenolic acids present in the medicinal plant exhibit strong antioxidant activity, which is dependent on their potential to form complexes with metal atoms, particularly iron and copper. This method is based on the principle of increasing in the absorbance of the reaction mixtures. The antioxidant activity rises in tandem with the absorption. The antioxidant compound present in the samples forms a colored complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride, which is measured at 520nm by a colorimeter according to the color of the reaction mixture.[15]

Antibacterial activity

The agar-well diffusion method was used to test the antibacterial activity of methanolic plant extracts. The agar-well diffusion method is commonly used to test plant extracts for antibacterial activity. The antagonistic activity of the extracts against different pathogenic bacteria was tested using the agar-well diffusion method. Plant extracts' antibacterial effectiveness is determined by the type and amount of phenolic content in the plant tissue, as well as the infection's innate resistance. In millimeters, the diameter of the created zone of inhibition can be measured.

Statistical analysis

Concentration and absorbance calculations were done by placing the constant values of m and c in the equation.

$Y = mx + c$

Where x = Conc. (µl/ml), m = Slope, and c = Intercept

Where one value is given, the other can be calculated, i.e., if the values of absorbance are given, then concentration values can be calculated from the above formula by placing the values of m and c as constant values.

The following equation was used to compute the percentage inhibition used to indicate the capacity to scavenge the DPPH radical:

DPPH scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$

Where A_0 denotes the control's absorbance and A_1 denotes the sample's absorbance.

Results

Phytochemical analysis

Phytochemical analysis of *Cannabis sativa* samples showed that the plant was rich in alkaloids, flavonoids, terpenes, steroids, and resins, showing positive results, whereas results for tannins, saponins, and volatile oil were negative, confirming the absence of these particular groups.

On the other hand, phytochemical analysis of *Murraya koenigii* samples showed that the plant was rich in phenol, flavonoids, tannins, alkaloids, and saponins, showing positive results. As shown in **Table 1** and **Table 2**.

Table 1. Results of phytochemical screening of the extract of *Cannabis sativa*

Extract constituents	Tests	Results
Alkaloids	Dragendroff's	Positive
Saponins		Negative
Terpenes & Steroids	Burchard	Positive
Tannins		Negative
Flavonoids	Lead acetate	Positive
Resins		Positive
Volatile oils		Negative

Table 2. Results of phytochemical screening of the extract of Murraya koenigii

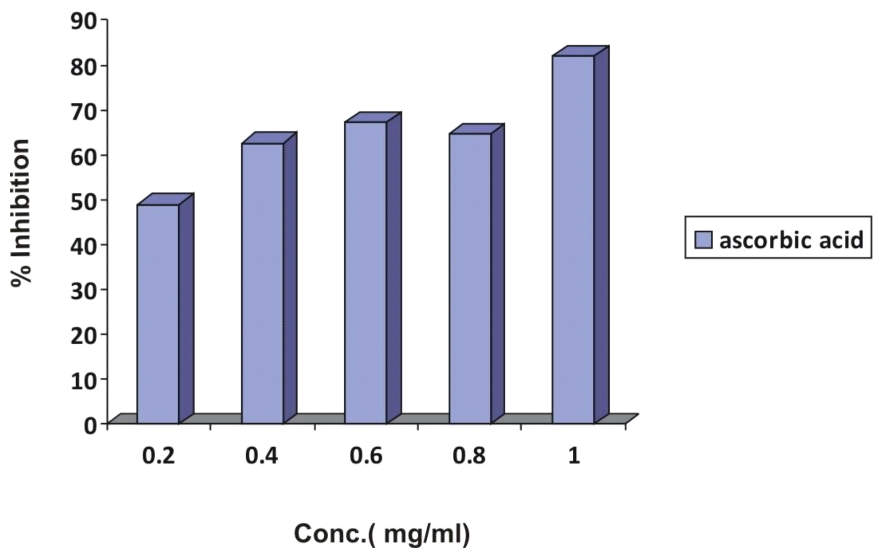
Extract constituents	Results
Phenol	Positive
Tannins	Positive
Saponins	Positive
Alkaloids	Positive
Flavonoids	Positive

Antioxidant activity

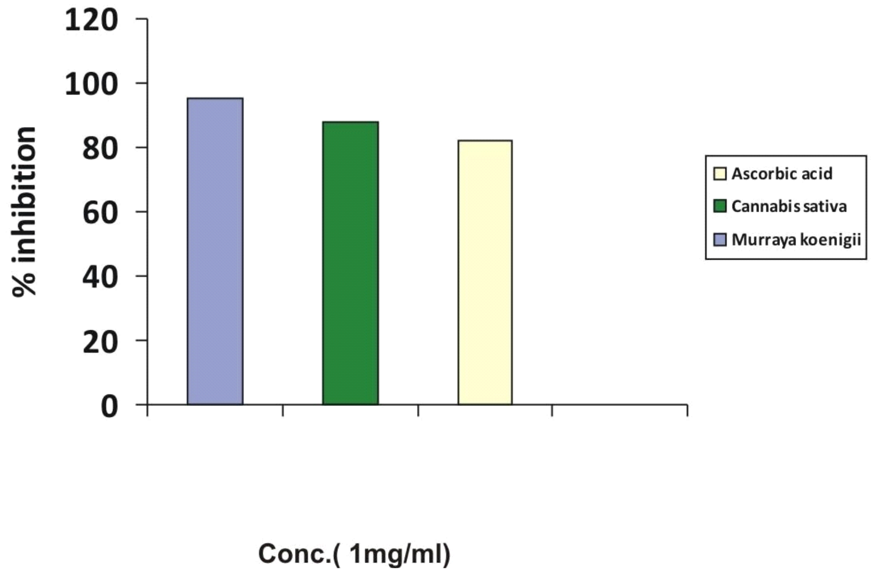
In this activity, the extract was measured by a DPPH radical scavenging assay. The results of this assay are plotted on graphs (**Figure 1**). The DPPH assay has a greater ability to scavenge free radicals, which shows more antioxidant activity. It was observed that with the increasing concentration of extract, the free radical scavenging activities also increased. In the present study, we observed that extract of *Murraya koenigii* leaves showed high inhibition as compared to *Cannabis sativa* in the case of DPPH radical scavenging activity, i.e., 95.32%, whereas *Cannabis sativa* showed low inhibition, i.e., 88.10%.

Figure 1. (a) DPPH free radical scavenging activity of ascorbic acid (b) DPPH free radical scavenging activity (1 mg/ml)

(a)



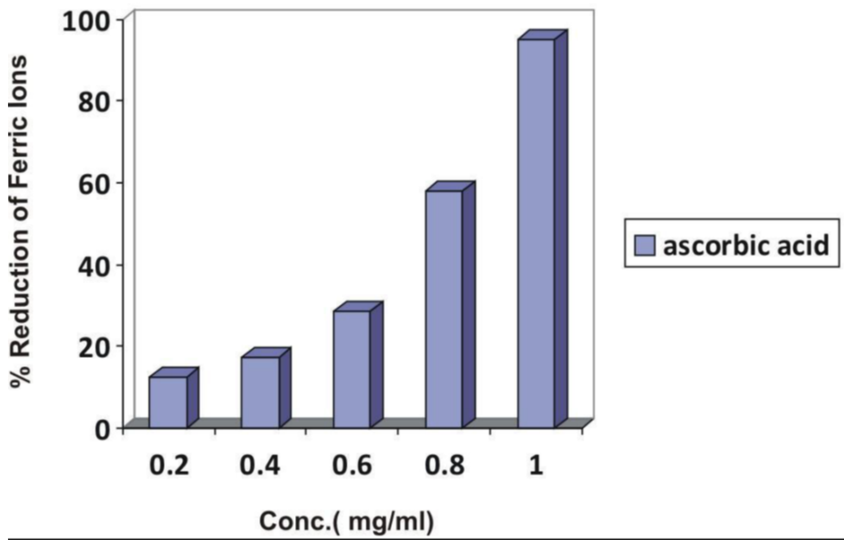
(b)



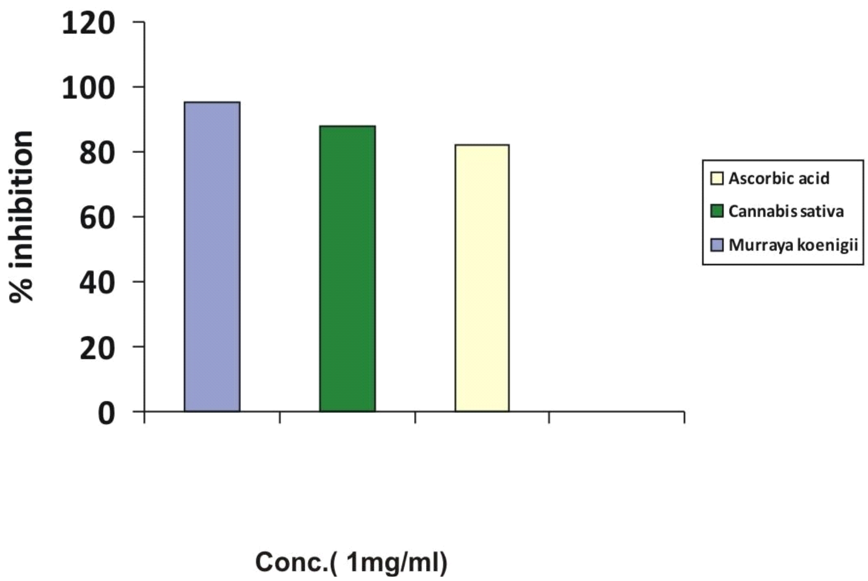
A Fe^{3+} to Fe^{2+} reduction assay was used to test the extracts' and fractions' lowering capacities. Depending on the content of antioxidants in the samples, the yellow hue changes to a pale green or blue tone. *Murraya koenigii* and *Cannabis sativa* extracts also had high levels of antioxidants such as phenolics and flavonoids. All of the samples demonstrated reducing capacity in a concentration-dependent manner, as assessed by a colorimeter at 520nm and displayed on graphs (**Figure 2**).

Figure 2. (a) FRAP: Ferric ions reducing capacity (%) of ascorbic acid (b) FRAP: Ferric ions reducing capacity (%)

(a)



(b)



A FRAP assay (ferric reducing antioxidant power assay) was also performed, which measures an antioxidant's reducing capacity. In this investigation, we discovered that *Murraya koenigii* extracts have a higher percent reduction of ferric ions (92.28%) than *Cannabis sativa* extracts (83.24%).

Antibacterial activity

As shown in **Table 3** and **Table 4**, the agar-well diffusion method was used to assess antibacterial activity. *Cannabis sativa* and *Murraya koenigii* methanolic extracts showed antibacterial action against a variety of stains *(E. coli, Salmonella typhimurium,* and *Staphylococcus pyogenes*). The highest activity, or zone of inhibition, was seen against *E. coli* (23 mm) and the lowest against *Staphylococcus pyogenes* (11 mm) in the case of *Murraya koenigii* leaf extract. In the case of *Cannabis sativa* leaf extract, the highest activity or zone of inhibition is observed against *Salmonella typhimurium* (22 mm), and the lowest activity or zone of inhibition is observed against *E. coli* (12 mm). As a control, DMSO is utilized, which shows no inhibitory zone.

Table 3. Antimicrobial activity of Murraya koenigii

Bacterial Strains	Control	20µl	40 µl	60 µl	80 µl	100 µl	200µl
Zone of inhibition (mm)							
Staphylococcus pyogenes	0	13	17	19	20	23	0
E.coli	0	11	14	16	17	19	0

Table 4. Antimicrobial activity of *Cannabis sativa*

Bacterial Strains	Control	20µl	30 µl	40 µl	50 µl	60 µl
Zone of inhibition (mm)						
Staphylococcus pyogenes	0	12	14	17	20	21
E.coli	0	15	16	17	19	22

Discussion

The extract of *Murraya koenigii* leaves had a higher percentage inhibition of DPPH radical scavenging activity than *Cannabis sativa*, i.e., 95.32% compared to 83.24%, but these results show high antioxidant activity. Also,curry (*Murraya koenigii)* leaf extract showed a higher percentage reduction of ferric ions (FRAP assay).

The extracts from both plants also showed antibacterial activity against different stains (*E. coli, Salmonella typhimurium,* and *Staphylococcus pyogenes*).

The phytochemical examination of *Murraya koenigii* revealed the presence of alkaloids, flavonoids, phenols, saponins, and tannins in this study. *Murraya koenigii* is anti-diabetic[16] and anticarcinogenic.[17] Saponin aids in the reduction of cholesterol and blood pressure. Many other review articles have highlighted the importance and phytochemical activity of *Murraya koenigii*. Many other studies have found medicinal effects from the essential oil prepared from *Murraya koenigii*.[18,19] Many of these phytochemical products are already in non-clinical trials against different diseases, but more research is still needed to isolate more such components to be used in other healthcare spheres.[20,21]

The leaf extract was subjected to a phytochemical examination to evaluate the presence of flavonoids, tannins, phenols, saponins, terpenes, steroids, resins, and volatile oils in the leaves of *Cannabis sativa*. Due to their physiological effects, such as their capacity to scavenge free radicals and possess anti-mutagenic, anti-carcinogenic, and anti-inflammatory properties, these phenolic classes have drawn a lot of interest.[22,23] Flavonoids, which have strong antioxidant properties, are the most prevalent phenolic chemical families found in plants.[24] Flavonoids are naturally occurring in plants and have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of anti-bacterial, anti-viral, anti-cancer, anti-mutagenic, anti-inflammatory, and anti-allergic activities.[25,26] The results show that the extract of cannabis shows the presence of alkaloids, flavonoids, etc. Thus, extracts possessing good free radical scavenging activity would be promising for the development as ingredients of functional foods and nutraceuticals, for example, with cancer-preventing or anti-aging properties. Including this study, many other studies have shown these results.[27]

These findings emphasize the relevance of conducting scientific studies to support ayurveda or other traditionally made medications in the development of future drug discoveries.

Conclusion

Murraya koenigii and *Cannabis sativa* extracts have shown high antioxidant activity. Both plants' extracts have antibacterial action against a variety of microorganisms. The findings highlight the importance of scientific research to support ayurveda in the development of future drugs.

Conflict of interest

The author declares conflict of interest due to being part of the editorial board at the Journal of Neoteric Life Sciences. To mitigate this conflict, the authors will abstain from participating in any decision-making processes related to this manuscript and agree to comply with any approach outlined by the Journal of Neoteric Life Sciences to manage this conflict.

Data availability statement

The data can be made available upon request from the author.

Ethics statement

Not applicable.

Acknowledgement

Not applicable.

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