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Phytochemical screening and evaluation of antioxidant potential and antimicrobial activity of *Muntingia calabura* Linn. leaf extract

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Abstract

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The emergence of various diseases mainly, noncommunicable diseases is a menace worldwide. Hence the plant-based medicines and drugs have gained attention recently. *Muntingia calabura* is one of the plants that possess anti-inflammatory, antioxidant, and pharmacological properties. Various parts of the plants, especially leaves, have various health benefits, which is still not explored completely. Hence the current study focuses on the phytochemical constituents, antioxidant and anti-microbial properties of *M. calabura* leaves. Identification of secondary metabolites which have potent antioxidant properties and medicinal value is much more essential for treatment of various health hazards. *M. calabura* possesses remarkable medicinal value, which warrants further and in-depth studies in the future. This plant is widely used in traditional medicine, in which almost all parts are utilized as it has the best antioxidant potential for cardiovascular diseases. The preliminary analysis of phytochemicals showed the presence of some beneficial phytochemicals such as terpenoids, flavonoids, tannins, and alkaloids. The results obtained by the determination of phenolics showed the highest concentration of phenolics in the aqueous extract. The results of total antioxidant activity clearly showed that the acetone extract has the highest antioxidant activity. Among various extracts, alcoholic extracts of 10 mg/ml and acetone extracts of 5 mg/ml and 10 mg/ml showed potential antibacterial activity against *E. coli* and in *Bacillus cereus*, alcohol and acetone extracts showed better zone of inhibition. The findings of this study provided the evidence of leaf extract possessing antioxidant activity and the presence of phytochemicals, exhibiting the potential for a wide range of therapeutic properties which can be used in manufacturing medicines and drugs.

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Abbreviations: DPPH: 2,2, diphenyl 1 picryl hydrazyl; MTCC: Microbial type culture collection and Gene bank; NSAIDs: Non-steroidal anti-inflammatory drugs; ROS: Reactive oxygen species; ANOVA: Analysis of variance; ET- Ethanol; AQ- Water; CH- Chloroform; AC- Acetone.

Introduction

In India and other parts of the world, infectious and non-infectious diseases have been rapidly increasing with an increase in their effects through time. India ranks second next to China in the world population, which creates more challenges to battle diseases, and this global issue has also led to the scope of finding cures for emerging diseases. Most people depend on allopathic medicines because of their effectiveness. But the main disadvantage of these kinds of medicines is that they tend to have the potential to cause side effects, which include diarrhea, dizziness, skin rashes, and drowsiness. On the other hand, herbal medicines comparatively have fewer to no side effects (Street et al., 2013). Not only does herbal medicine treats diseases from their causes, but it also provides numerous health benefits to the human body (Salih et al., 2018). The therapeutic properties of plants have been evaluated and transformed into useful outcomes in the form of medicines and drugs for the human race (Yu et al., 2021). One such plant that possesses multi-therapeutic properties is *Muntingia calabura* (Mahmood et al., 2014). It is very abundantly grown throughout the regions of India (Pereira et al., 2018). But unfortunately, this plant is not much appreciated and acknowledged for its pharmaceutical uses, mainly because very few studies have been carried out to study and understand the plant and its contribution to the field of medicine, as discussed by Mahmood et al. (2014). Only a handful of medicinal plants prove to contain therapeutic properties in all the parts of the plant, and *M. calabura* is one of them as its leaves, bark, flowers, and fruits have a broad range of pharmaceutical properties, which include anti-inflammatory (Preeti et al., 2012), antimicrobial (Sufian et al., 2013), antioxidant (Pereira et al., 2018), anti-fungal, anti-diabetic (Preeti et al., 2010), antipyretic (Zakaria et al., 2007), antiulcer (Zakaria et al., 2014) and anticancer (Nazir et al., 2017) activities.

The boiled leaves were used as a remedy for the treatment of gastric ulcers and traditionally, they were also taken to relieve headaches and colds (Zakaria et al., 2014). The color of fruits varies from red to yellow depending on the variety of plants (Nasution et al., 2022) and these fruits mainly possess anti-bacterial (Simamora et al., 2020) and antioxidant properties (Preeti et al., 2010). In some studies, it has been found that they also contribute to immunostimulatory activity (Sujono et al., 2021). Not only do the leaves, flowers, and fruits have these therapeutic properties, but the

barks of this plant also have similar antihyperuricemics activity (Safrida et al., 2019). Interestingly, in some parts of the world, the bark is boiled and taken to relieve swelling in small extremities (Mahmood et al., 2014).

Based on the literature carried out, this plant had limited traditional uses, but still in some countries such as Peru, the bark and flowers were used as an antiseptic and to reduce the pain. In Colombia, the infusion of the flowers is used as a tranquillizer and tonic. In Mexico, measles and stomach pain were treated using *M. calabura* plant. In the Philippines, the flowers were used to treat headache, or as tranquillizers and antidiyspeptics. In different regions of Malaysia, the *M. calabura* roots have been used as an abortifacient in Malaysia (Mahmood et al. 2014). The fruits are either processed in to jams or sometimes eaten fresh and the leaves can be used for preparing tea (Nirmala et al. 2020).

Inflammation is the body's immunological response to trauma and infection. Inflammation can be sometimes critical and painful, and it can even damage the tissue to a greater extent. The treatment for inflammation is usually done with NSAIDs (Non-Steroidal Anti-inflammatory Drugs). These NSAIDs are found to exhibit allergic reactions in many individuals. Along with that intake of these drugs can cause problems like indigestion, stomach ulcers, drowsiness, etc. When these are taken for a prolonged period it can lead to kidney, liver, and even heart problems. So, there is a need for an alternative which have fewer and no side effects along with its efficacy. *M. calabura* as mentioned is a multi-therapeutic plant having effective anti-inflammatory property in treating rheumatoid arthritis.

Numerous researchers have examined the various bioactive and pharmacological properties of *M. calabura*. In one of the recent studies, the antihyperglycemic properties of *M. calabura* were noted by Gunny et al. (2024). The plant also possesses the anti-inflammatory, anti-analgesic, anti-hypertensive, and gastro-protective properties as reported by GKM (2023). Pertiwi et al. (2020) stated that the leaf extracted in 96% ethanol exhibited the highest total antioxidant activity, reducing power and DPPH radical scavenging activity. Sinaga et al. (2022) found that in the ethanolic extract of the leaves of *M. calabura* had a high antioxidant activity. The secondary metabolites or the phytochemicals, produced in a plant is the reason for

many of its therapeutic properties. The main objective of this study was to evaluate the phytochemical constituents, antioxidant and anti-microbial properties of *M. calabura* Linn leaves. Identification of secondary metabolites which have the potent antioxidant property and medicinal value is much essential for treatment of various health hazards.

The main objective of this study was to conduct a thorough evaluation of *M. calabura* leaves by analyzing its phytochemical composition and assessing its antioxidant and antimicrobial properties. For this, four different solvents (ethanol, water, acetone, and chloroform) were used for preparing the extract of the leaves. A comparative analysis was conducted to determine which organic solvent extract contains the highest concentration of phytochemicals and exhibits the most significant effects. Thus, this study aims to provide insights into the medicinal value of *M. calabura* and its potential applications in phytomedicine. It not only contributes to the understanding of *M. calabura*'s role in health boosting, but also supports the development of natural, plant-based alternatives for improving the human health.

Materials and methods

Sample collection

The plant *M. calabura* is one of the most widely growing trees, hence it was easy to find and identify it. The leaves were picked up by hand and stored in a clean polythene bag and were used for the experimentation on the same day. While collecting the leaves, mature leaves were preferred as they would contain more secondary metabolites. Two trees were identified and were used as the primary source of leaves for experimentation. The leaves were collected from the trees located in Kannada Bhavan, JC Road, Bangalore, Karnataka.

The location of the trees are - [https://goo.gl/maps/4KtA3R9SmzhCQtXq612°57'47.3"N 77°35'05.5"E](https://goo.gl/maps/4KtA3R9SmzhCQtXq612°57'47.3) and [https://goo.gl/maps/egUUZ4TAVP1qMWZe7 12°57'43.2" N 77°35' 08.0"E](https://goo.gl/maps/egUUZ4TAVP1qMWZe7 12°57'43.2). The studied plant was sent for specimen identification to Herbarium and the plant was identified by Dr. Noorunnisa Begum, Curator, Foundation for Revitalisation of Local Health Traditions, Yelahanka, Bangalore-560064, Karnataka, India and the voucher specimen number is FRLHT Acc. No. 6107. The plant identified was *M. calabura* belonging to family Muntingiaceae.

Extract Preparation: Wet and dry extracts of the leaves were prepared. In both the processes, the leaves were weighed and then washed with water and then extracted in the solvent as per the methodology by Tlili et al. (2019).

Wet extract: The washed leaves were grinded in mortar and pestle for ten minutes until a homogenate is formed. Different solvent systems were tried for the extraction process such as water, acetone, chloroform and ethanol. The solvent system used while grinding and after completion, we measured the volume of the extract and solvent was added to make up to 10X the weight of the leaves by volume. The solution was then filtered using a filter paper and the filtrate was labelled as the wet extract. For one gram of sample, 10 ml of solvent was used for extraction and extraction was done for a time period of ten to fifteen minutes till a homogenate was formed. Once the extraction is completed, it is filtered and the supernatant obtained is used for further phytochemical and quantitative analysis.

Dry extract: Fresh leaves collected were weighed and sun dried for five days. Once it is completely dried, weight of the sample is measured and further powdered using a blender. 1 gm of grinded leaf extract was taken and dissolved in ethanol (solvent). After the completion of 24 hours, the mixture was boiled until the solvent completely evaporated and collected the leftover powder. This powder was transferred to a clean test tube and 25 ml of fresh solvent was added and filtered. This was further used in the qualitative and quantitative analysis.

Phytochemical analysis

Phytochemical analysis is a technique used to identify and quantify the various chemical compounds present in plant materials. In pharmacognosy, the study of medicinal plants, phytochemical analysis is used to identify and characterize the active ingredients in a plant that are responsible for its medicinal properties. This is important because the active ingredients in a plant can vary depending on a variety of factors, such as the species of the plant, the location where it is grown, and the time of year it is harvested. Wet extracts were used for phytochemical analysis. Terpenoids, alkaloids, steroids, flavonoids, tannins extract, saponins and glycosides were tested following standard protocols (Godghate and Sawant, 2013; Egwaikhide and Gimba, 2005).

Quantification of phenolics

For the quantification of phenolics, 1 gram of leaf sample was extracted in all four different solvents such as 100% water, acetone, ethanol and chloroform (volume of 10 ml) for ten to fifteen minutes as per methodology adopted by Menon and Rao (2012) and Oluwatoyin et al. (2020). The extracts were prepared using 1000mg of wet extract and 100 mg of dry extract. A standard graph with catechol reagent was made following the Folin-Ciocalteu (FCR) method and then analyzed for the phenolics content in the crude sample using the data in the standard graph. The working standard catechol reagent was prepared by diluting the standard solution 10X with distilled water. Working standard and standard solution were covered with foil as catechol is a light sensitive chemical. Each tube was made up to 5 ml using distilled water and 0.5 ml of 50% Folin-Ciocalteu [FCR] reagent was pipetted into each tube. The entire tubes were left to incubate at room temperature for 3 minutes and then 2 ml of 10% Na₂CO₃ was added to every tube. All tubes were kept in the water bath for two minutes and the blue color developed was measured for its absorbance at 620 nm using a colorimeter.

Total antioxidant activity

Antioxidant activity was determined using the methodology by Menon and Rao (2014) by DPPH method. One gram of the sample was extracted in 100% of 10 ml of different solvent systems such as ethanol, water, acetone and chloroform. 20 μ l of the extract was taken in 6 test tubes, where three tubes, were considered as test samples and the remaining three tubes samples were considered as control. 880 μ l of methanol was added into all the test tubes and 1ml methanol was taken separately as blank. One millilitre of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (0.1 mM) was dissolved in methanol, added to the tube, and shaken vigorously. After the addition of DPPH solution, all of the test tubes were shaken gently and allowed to stand at 27 °C in a dark place for 30 min. Then 1.5 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent was added to the remaining three tubes and marked as test samples. After the incubation period, 1.5 ml of DPPH reagent was added to the control test tubes, and the absorbance was read at 517 nm. The percentage of antioxidant activity was calculated using the formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Antimicrobial activity

The antibacterial effect was evaluated on bacterial strains namely *E. coli* (MTCC-739) and *Bacillus cereus* (MTCC-1369) bacterial species were used during the study. The test microorganisms used for the current study were *E. coli* which is a gram-negative rod-shaped bacteria and *Bacillus cereus* which is a gram-positive bacteria. The tested strains were obtained from the Department of Microbiology, Indian Academy Degree College, Bengaluru, cultured in nutrient broth and stored at -20⁰C as a glycerol stock.

Among various methods used for antibacterial activity, the agar well diffusion method was the best method for accurate results (Mustafa et al., 2007, Sarita et al., 2019, Mahuya De Ghosh et al., 2020). This method was used to screen the antibacterial activities of different samples namely leaves, roots, bark and petals. The antibacterial effect was evaluated on gram positive and a Gram negative bacterial strains namely *Bacillus cereus* (MTCC-1369) and *E.coli* (MTCC-739). Nutrient agar was sterilized and poured into sterile plates. After solidification, the agar plate surface is inoculated by spreading 0.1 ml of overnight-grown microbial inoculum. Then a hole of 7 mm diameter is punched aseptically with a sterile cork borer to form the wells. Four holes are punched, one for a positive control, one for a negative control, and two for extracts of different concentration (5 mg/ml and 10 mg/ml). Ampicillin of 100 μ g/ml was used as a positive control, and respective solvents were used as a negative control to determine the sensitivity of the strains. All samples (one gram of sample extracted in 10 ml of solvent (100 mg/ml) are loaded into the wells at a constant volume of 20 μ l (Anupam Ghosh et al., 2008, Mahuya De Ghosh et al., 2020; Akhter Ali Siddiqui and Rizwana Begum, 2021).

The plates are pre-incubated in refrigerator for 30 minutes to allow uniform diffusion of samples into agar then shifted to incubator at 37⁰C for 18 h (Mustafa et al., 2007; Anupam Ghosh et al., 2008). The antimicrobial agent diffuses into the agar medium and inhibits the growth of the microbial strain tested. After the completion of incubation period, zone of inhibition appeared around each well which was measured and recorded to the nearest size in millimeters and later converted to centimeteres. All of the experiments were performed in triplicate, and results were expressed as diameters (mm) of inhibition (Atikya et al., 2014; Dilshad et al., 2021; Kıymet et al., 2005).

Statistical analysis

All the data were subjected to statistical analysis using SPSS software. The triplicate data were subjected to One-Way ANOVA, and the $P < 0.05$ were calculated.

Results and discussion

Antioxidant activity

Antioxidants are known to scavenge the free radicals that are produced in plants and animals. An analysis was carried out to determine the antioxidant activity of the multi-therapeutic plant, *M. calabura*. The DPPH method was used to evaluate the antioxidant activity of the leaf extract, which was extracted using four different solvents (acetone, chloroform, ethanol and water). The antioxidant activity of all four solvents was compared, and it can be observed from the figure that, the acetone extract of *M. calabura* leaves showed highest antioxidant activity (358.33%) and was statistically significant ($P < 0.05$), P value = 0.046 while the water extract showed only negligible percentage of antioxidant activity (12.5%). The chloroform and ethanol extract also exhibited significant levels of antioxidant activity ($P < 0.05$), when compared to the water extract, as depicted in Fig. 1.

The DPPH assay is one of the most convenient and affordable methods to easily determine the antioxidant activity of any plant extract, which is based on the donation of a hydrogen atom by the antioxidants to the nitrogen atom of DPPH that gets reduced. The amount of DPPH reduced is correlated to the scavenging activity of the antioxidants. In the present study, the antioxidant potential of *M. calabura* leaf extract was determined using the DPPH method and the results were precisely analysed. The investigated results showed that the leaf extract of the plant possessed antioxidant properties. In a comparative analysis among the four solvent extracts, acetone extract showed greater antioxidant activity. Similar results were obtained in Malaysian cherry leaves by Pungot et al. (2020) and *M. calabura* leaves by Pertiwi et al. (2020) related to the antioxidant activity. Testing with DPPH is based on reducing radical compounds that are purple to yellow due to the transfer of hydrogen atoms by antioxidant compounds so that DPPH radicals become stable (Floegel et al, 2011; Wootton-Beard and Ryan, 2011).

Total antioxidant activity is a measure of determining

the overall antioxidant potential of the plant. It can be positively correlated with the phytochemicals and phenolic content in the current study. The antioxidant activity may also come from the presence of secondary metabolites that potential as antioxidant agents, such as volatile oils, carotenoids, and vitamins. The antioxidant activity of phenolic compounds is primarily due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen (Indirayati et al. 2020).

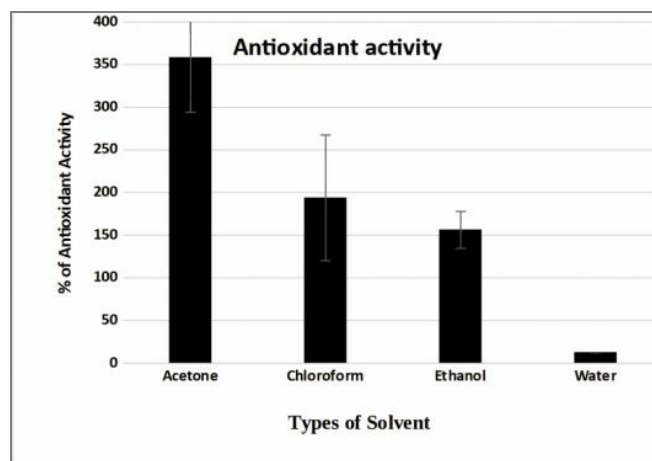


Fig. 1: Determination of antioxidant activity of *M. calabura* leaf extract in four different solvents.

Phytochemical analysis

Preliminary analysis for the presence of phytochemicals in the *M. calabura* plant was carried out. For the leaf extraction, four different solvents (acetone, chloroform, ethanol and water) were used, so a comparative analysis was made. The choice of solvents is important to determine the polarity of constituents, especially phytochemicals, in a plant. The results, as given in Table 1 showed the presence of terpenoids, flavonoids, tannins, reducing sugar, steroids, and phenols in all four of the extracts. Alkaloids were present in all the extract except water and the presence of anthraquinone was solely detected in ethanolic extract. However, the ethanolic extract showed better results for all the phytochemicals mainly phenols and anthraquinones. The positive testing of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Based on the results obtained, it was concluded that ethanol can be selected as best solvent for extraction of secondary metabolites. Similar results were observed in fruits of same plant in methanolic extract (Ariffin et al., 2022), and in the

ethanolic extract of leaves as well as fruits (Buhian et al., 2016). According to Tiwari et al. (2011) different phytochemicals can be examined and screened based on the best solvent used for the extraction procedure. The phytochemicals best extracted in polar or non-polar solvents in the current study, based on qualitative

tests, help to determine the efficacy of the leaves and plant. The presence of these phytochemicals can be correlated with the best antimicrobial and antioxidant potential of the *M. calabura* leaves as per the previous research (Sari et al., 2020; Ariffin et al., 2022; Muniyappan et al., 2022).

Table 1. Qualitative analysis of phytochemicals in the different solvent extracts of *M. calabura* leaf.

Phytochemicals	Solvents			
	ET	AC	AQ	CH
Terpenoids	++	++	+	+
Flavonoids	+	+	+	+
Tannins	++	++	+	+
Alkaloids	+	+	-	+
Reducing sugar	++	++	++	+
Anthraquinones	+	-	-	-
Steroids	+	+	+	+
Phenols	++	++	+	+

‘+’ denotes present; ‘++’ denotes present with highly colored/dark color; ‘-’ denotes absent; ET- ethanol, AC- alcohol, AQ- water, CH- chloroform.

Each one of the above-mentioned phytochemicals has its own therapeutic importance and mechanism of action. For example, flavonoids show very good antioxidant and antimicrobial activity, whereas anthraquinone which is a photodynamic compound possesses antiviral, anticancer and a broad spectrum of medical action especially in immunological disorders (Mantareva et al. 2024). Several studies have evaluated the uses and potential of the phytochemicals and provided evidence that they could fight chronic diseases, especially cardiovascular disease (Pertwi et al. 2020; Sinaga et al. 2022; Dhawan and Gupta, 2017).

Total phenols

Phenolics are also known to be secondary metabolites that consist of a benzene ring and one or more hydroxyl groups attached to it. For the quantification of phenolics, Folin-Ciocalteu method was followed. The results were drawn from the colorimetric estimation, which showed the presence of a large amount of phenolics in the water extract (0.07776 mg/g) whereas the alcohol extract showed the presence of least amount of phenolics (0.0216 mg/g) as depicted in Fig. 2. P value was found to be $P = 0.037$. Generally, phenolics are extracted and quantified to their maximum in alcoholic medium, but our results were in contrast with the other studies. This variation may be due to the environmental stress and other climatic variations that affected the secondary metabolite accumulation in *M. calabura*.

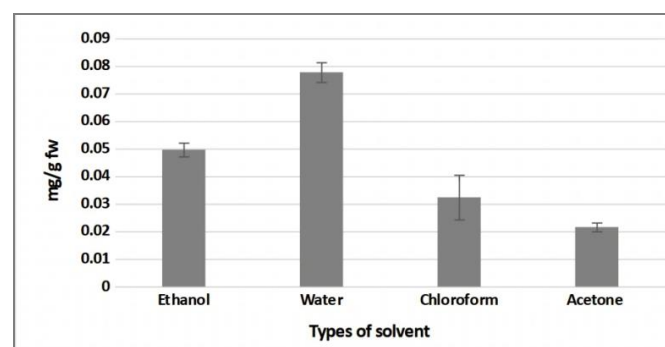


Fig. 2: Determination of phenolics of *M. calabura* leaf extract in four different solvents.

In the present study, the presence of phenols in the preliminary screening paved way for the quantification of phenols, as they are a huge group of phytochemicals with numerous beneficial properties. The results showed the presence of phenolic compounds in all the four solvent extracts, but aqueous extract showed good amount of the phenolics. Several studies such by Menon and Rao (2014); Menon and Rao (2018) have suggested that phenols possess the free- radical scavenging antioxidant property and are mainly involved in the defence mechanism of the plant against any foreign particles. Phenols are also the major contributors for the antioxidant property, and in some of the studies, the total phenolics and total antioxidant activity are related to *M. calabura* (Adam et al., 2021).

Moreover, phenolics are induced by natural defence

stimulators in different amount depending upon the plant. The binding of the active molecule to receptor of plant leads to numerous chemical reactions and synthesis of phenols (A. L. N'CHO et al. 2023) that further contribute to the defence mechanism in *M. calabura* and resist its pathogenic attack. The anti-inflammatory activity of the *M. calabura* is mainly due to the abundance of phytochemicals especially phenols, alkaloids, tannins etc. as stated by Rezeki et al., (2023). Studies have also suggested that phenols act as anticancer, antiaging and anti-inflammation molecules (Pereira et al., 2018; Pratiwi and Dewi, 2022; Rezeki et al. 2023).

Antimicrobial activity

It is fundamental to develop a better understanding of the antimicrobial mechanisms of plant crude extracts against spoilage and pathogenic microorganisms. Based on the activity performed with two bacterial species, *Bacillus* and *E. coli* in the current study, it was observed that a higher concentration of ethanolic extract could inhibit the bacterial zone compared with water extract. The inhibitory effect was maximum in ethanolic extracts as it could suppress the microbial growth. The results depicted that high concentrations of alcoholic extract (10mg/ml) and both the concentration of acetone extract (5 and 10 mg/ml) showed good antibacterial activity against *E.coli* (Fig. 3), and in the case of *Bacillus cereus* apart from alcohol and acetone, chloroform extract has shown mild inhibition (Fig. 4). However, the water extract resulted in no inhibition against neither of the bacterial strains. A two-way ANOVA between the different solvent extracts and the strains was conducted to compare the antimicrobial activity. There was a significant effect of the strain on antimicrobial activity at the $p < 0.05$ level. The significance effect of the strain's activity response was reported to be [$F = 7.31422$, $p = 0.02421$]. P value is 0.0242.

Understanding the antimicrobial action of plants like *M. calabura* is the first step in the best utilization of these extract as natural antimicrobial agents which can be further studied to be used to extend the shelf life and maintain the food quality of certain preserved foods (Gonelimali et al., 2018). This also helps us to make use of the potential extracts in synthesis of ointments and other pharmacological products (Tamakou et al., 2011). Evaluation of antibacterial activity of the plant extracts against *Bacillus cereus* (MTCC-1369) and *E. coli*

(MTCC-739) using agar well diffusion technique was carried out. Among various extracts, alcoholic extract of 10mg/ml and acetone extract of 5 mg/ml and 10 mg/ml showed potential antibacterial activity against *E. coli* (Fig. 3) and in case of *Bacillus cereus* alcohol and acetone extracts showed better zone of inhibition than chloroform extract, which has shown mild inhibition (Fig. 4). Ampicillin of 100 $\mu\text{g/ml}$ was used as positive control in the study, and the respective solvent as negative control. The results revealed that all plant extracts were potentially effective in suppressing microbial growth of both gram positive and gram-negative strains, but the water extract resulted in no inhibition against neither of the bacterial strains.

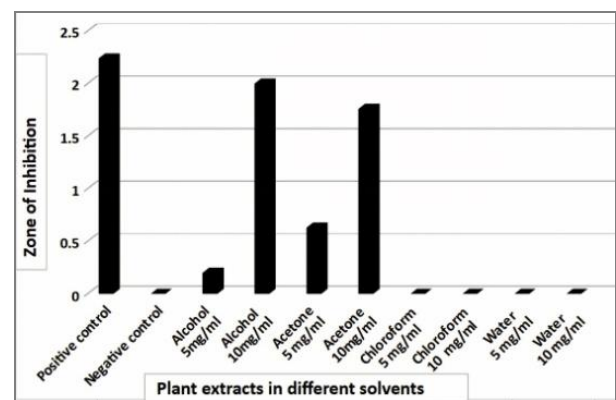


Fig. 3: Antimicrobial activity of *M. calabura* leaf extract against *E. coli*.

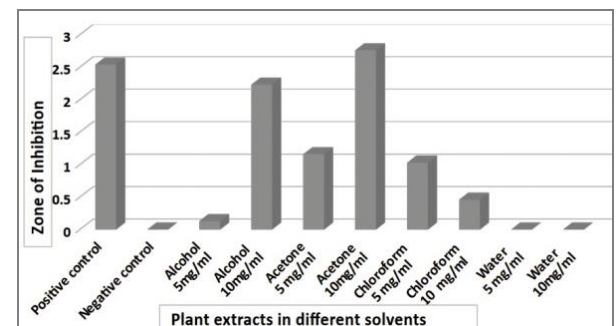


Fig. 4: Antimicrobial activity of *M. calabura* leaf extract against *Bacillus cereus*.

Some of the studies were performed using *M. calabura* methanolic extract against *Staphylococcus aureus*, *Salmonella typhimurium*, and *Shigella sonnei* using the disc diffusion and broth dilution methods and the extract of *M. calabura* leaves were the most successful when tested against *S. aureus* especially (Cheong et al., 2022). Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the

solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. The polarity, from least polar to most polar, of a few common solvents is as follows: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water (Altememi et al. 2017).

Plant extracts can have antimicrobial activity against bacteria, fungi, viruses, and protozoa. It is fundamental to develop a better understanding of the antimicrobial mechanisms of plant crude extracts against spoilage and pathogenic microorganisms. The antibacterial activity is attributed to the presence of phytochemicals such as flavonoids, tannins, saponins, and phenolic compounds. These compounds have been linked to the antimicrobial properties observed in *M. calabura* leaves (Mosa et al., 2022). The solvent extracts are studied for its effect against resistant microbial strains and could be used as natural preservatives to control food poisoning. Some researchers believe that plant extracts can disrupt the microbial cell membrane, causing cell death. Others believe that the extracts' hydrophobicity allows them to react with the protein of the microbial cell membrane, changing its structure and permeability (Mosa et al., 2022). The leaves of *M. calabura* represent a promising natural source of antimicrobial agents, with potential applications in developing alternative treatments for infections caused by antibiotic-resistant bacteria (Cheong et al., 2022). Their rich phytochemical profile not only supports their use in traditional medicine but also highlights their relevance in modern pharmacological research aimed at addressing the growing issue of antimicrobial resistance (Sowjanya et al., 2023).

Conclusions

The contribution of plants to therapeutic aid has been massive to the field of drug and health care. This is mainly due to the presence of antioxidants, metabolites and phytochemicals such as terpenoids, flavonoids, tannins, alkaloids, phenols, steroids etc. All these phytochemicals have more than one application and their negligible side effects, unlike other synthetic chemicals, have increased the usage of medicinal plants in the production of new drugs and medicines. In the present study, antioxidant activity is determined using DPPH assay. The results gained by this analysis showed good percentage of antioxidant activity with the acetone leaf extract, which provides a simple data that makes it

possible to produce a herbal drug with the potential of antioxidant activity. Phytochemicals not only help in the normal biological activities of the plant; they also help in the defense mechanism of immune system when given in the form of medicine to human beings. Further, the preliminary analysis of phytochemicals and quantification of phenolics of *M. calabura* plant extract showed the potential to act as a good source for the production of various useful drugs especially anti-inflammatory drugs which can reduce the inflammation in rheumatoid arthritis. The data based on antioxidant phenols and antioxidant activity, supports the hypothesis of anti-inflammation mediation which can further be confirmed by In-vivo studies using carrageenan induced inflammation model.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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