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**EVALUATE THE ANTIOXIDANT ACTIVITY OF THE METHANOLIC
LEAF EXTRACTS OF *Murraya paniculate*, *Trachyspermum ammi*, *Carica
papaya* BY DPPH ASSAY**

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ABSTRACT

This analysis aims to determine the antioxidant activity of a methanolic extract of *Murraya paniculate* leaves. (*M. paniculate*), *Trachyspermum ammi* (*T. ammi*), *Carica papaya* (*C. papaya*) using 1,1-diphenyl-2-picrylhydrazine (DPPH). The antioxidant activity of methanolic extract of *T. ammi*, *M. paniculate* and *C. papaya* leaves was assessed using the DPPH free radical assay, which is one of the most accurate methods for determining antioxidant activity. The free radical scavenging activity of the methanolic leaf extract of *T. ammi*, *M. paniculate* and *C. papaya* increased with increasing concentration, indicating that it has antioxidant properties. The EC₅₀ value of methanolic extract of *T. ammi*, *M. paniculate* and *C. papaya* shows EC₅₀ of 47, 46, 45 µg/mL respectively. Ascorbic acid was used as control. This research indicates that *T. ammi*, *M. paniculate* and *C. papaya* extracts have a lot of antioxidant capacity and may be useful for both nutritional and medicinal purposes.

**Keywords: Antioxidant, *Murraya paniculate*, *Trachyspermum ammi*, *Carica papaya*,
DPPH, Analysis**

INTRODUCTION

Aromatic plants are also commonly used as dietary supplements [1]. Their importance as a natural antioxidant source [2] is particularly intriguing. Because of the rising safety issues associated with the consumption of synthetic antioxidants, researchers are looking for cheaper and safer antioxidants from natural sources, especially plants. Polyphenols are the most common plant compounds with antioxidant activity. Polyphenols' antioxidant activity is attributed to their redox properties [3], which include the ability to absorb and neutralize free radicals, quenching singlet and triplet oxygen, and decompose peroxides. Flavonoids are the most abundant and widely distributed phenolic compounds in plants. These are found in most plants and are thought to protect against free radical-related damage in a variety of ways, including direct scavenging of free radicals and inhibition of free radical-producing enzymes [4].

In the pathogenesis of many chronic diseases, oxidative stress is a significant risk factor. Free radicals and other reactive oxygen species are known to play a role in the pathogenesis of diseases like asthma, inflammatory arthritides, diabetes, Parkinson's and Alzheimer's disease, cancer, and atherosclerosis. Human aging is also said

to be caused by reactive oxygen species [5, 6]. Any material that delays or inhibits oxidative damage to a target molecule is considered an antioxidant [7]. The ability of an antioxidant to trap free radicals is its most important feature. Antioxidant compounds such as phenolic acids, polyphenols, and flavonoids scavenge free radicals including peroxide, hydroperoxide, and lipid peroxy, inhibiting the oxidative processes that trigger degenerative diseases [8]. Since ancient times, herbal plants have been thought to be healthy antioxidants.

The antioxidant function of ethanolic extracts of *murraya paniculate*, *trachyspermum ammi*, and *carica papaya* plant leaves was investigated in vitro in this study. These plants are commonly used as medicinal plants in Tamilnadu. *Carica papaya* (*C. papaya*) belongs to the Caricaceae tribe. Many Caricaceae species have been used to treat a variety of ailments [9]. The papaya plant is grown in the tropics and subtropics in countries such as India, Brazil, Indonesia, Mexico, and Nigeria, with India producing the most papaya. The leaves have been used in India for colic, flu, beriberi, abortion, and asthma. In addition, papaya leaves have long been used to treat jaundice, malaria, and dengue fever [10, 11].

The dried leaves are used as a blood purification tonic. *C. papaya* leaf tea is used to treat obesity and is also beneficial in the treatment of chronic indigestion, high blood pressure, and arteriosclerotic heart disease [12]. Active compounds found in papaya leaves include papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides, and glucosinolates, which have been shown to have antioxidant activity and reduce lipid peroxidation.

Murraya is a genus of 14 species in the rutaceae family. *Murraya paniculate* (*M. paniculate*) L. Jack is a small tropical evergreen shrub that grows in tropical and subtropical climates [13]. *M. Paniculate* has been used as a medicinal plant and in herbal medicine for many years. Extracts from this plant's bark and leaf have been shown to have antioxidant, anti-inflammatory, antidiabetic, antimalarial, antidiarrheal, antifungal, and antibacterial properties in previous studies. The bioactive ingredients in *M. paniculata* have been studied, including coumarins, indoles, alkaloids, phenolic acids, flavonoids, and terpenoids. These compounds serve as scientific justification for *paniculata*'s common use [14]. *Trachyspermum ammi* (*T. ammi*) belongs to the Apiaceae family, which plays an

important role in pharmaceuticals and the food industry. It is widely used as a flavoring ingredient in several fields and as a traditional folk remedy. Antispasmodic, carminative, and stimulant properties are among the plant's many functional characteristics. It has long been used to treat diarrhea, atonic dyspepsia, masses, and stomach tumours, as well as abdominal pains and lack of appetite, as well as bronchial disorders, asthma, galactagogue, and amenorrhea [15].

MATERIALS AND METHODS

Plant materials and Preparation of plant extract

The dried leaves powder of *T. ammi*, *M. paniculate* and *C. papaya* were collected from Siddha and Ayurvedic shops. The 50 g of each leaves powder was extracted by 98% methanol solvent and filtered using Whatman filter paper. The obtained *T. ammi*, *M. paniculate* and *C. papaya* filtrates were concentrated under vacuum in a rotatory evaporator at 40°C and stored at 4°C for further antioxidant activity.

Antioxidant activity test

After weighing 50 mg of crude methanolic extract, each extract was dissolved in 50 mL methanol, yielding 1000 g/ml. Using sufficient dilution, different concentrations (75, 100, 250, 500, 750, 1000

µg/ mL) of extract working solutions were prepared from the stock solution. The antioxidant activity of the plant extracts and the standard ascorbic acid was determined by using 2, 2-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich) to test the radical scavenging effect [16]. In methanol, the test extracts' diluted working solutions were prepared. In 75, 100, 250, 500, and 1000 µg/ mL solution. ascorbic acid was used as a normal. In methanol, 0.002% DPPH was prepared, and 1 mL of this solution was mixed separately with 1 mL of sample solution and 1 mL of normal solution. The optical density of these solution mixtures was calculated at 517 nm using a UV visible spectrophotometer after 30 minutes in a dark space (Perkin Elmer). As a blank, 1 mL of methanol with 0.002% DPPH solution (1 mL) was used. The optical density was measured, and the percent inhibition was determined using the following formula.

$$\text{DPPH scavenging effect (\%)} = \frac{A-B}{A} \times 100$$

A = Optical density of the blank, B = Optical density of the sample

RESULT AND DISCUSSION

Table 1 (optical density) and **Table 2** (DPPH scavenging activity) show the antioxidant characteristics of methanol extracts of the tested plants as determined by DPPH scavenging activity (antioxidant

inhibition efficiency). Natural antioxidants and their health benefits have received a lot of attention in recent years. Many complex diseases are treated and prevented with antioxidant-based drug formulations. Plants are a major source of natural antioxidants, and they contain a variety of antioxidative secondary metabolites with therapeutic potential. Polyphenols are the most common antioxidant compounds found in plant matter. Their antioxidant activity stems from their redox properties, which make them useful as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators, and ferric hemoglobin reductants. The existence of reductants, which act as antioxidants by breaking the free radical chain by contributing a hydrogen atom or by preventing peroxide formation, is generally associated with reducing capacity. Flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans, and lignins are common phenolic compounds found in medicinal plant tissues. Antioxidant activity is one of the biological effects of these compounds [17].

The free radical scavenging behavior of methanolic leaf extracts of *T. ammi*, *M. paniculate*, and *C. papaya* was determined using the radical scavenging effect on DPPH as a starting point. DPPH has the advantage

of being unaffected by certain side reactions caused by various additives, such as metal ion chelation and enzyme inhibition, which distinguishes it from laboratory-generated free radicals like the hydroxyl radical and superoxide anion, which can be affected by side reactions. When an antioxidant is present in the medium, the colour of a freshly prepared DPPH solution changes from deep purple to yellow. Antioxidant molecules react with DPPH, causing the free radical to be scavenged by hydrogen donation. The absorbance of the DPPH radical at wavelength 517 nm decreases as it is scavenged. At various concentrations, methanolic extracts of *T. ammi*, *M. paniculate*, and *C. papaya* leaf demonstrated antioxidant activity comparable to regular ascorbic acid (**Figure 1, Figure 2 and Figure 3**). The percentage antioxidant activity increased dose-dependently at all concentrations measured. The tests were done in triplicate in each concentration, and the mean SD of the three tests was taken.

The extract of the *T. ammi* shows highest 66.762, 70.6, 75.1, 83.7, 90.1, 94.7 % antioxidant activity (Inhibition efficiency) at 75, 100, 250, 500, 750, 1000 µg/mL respectively (**Table 2 and Figure 1**). Similarly, *M. paniculate* shows 71.9, 75.3, 79.9, 83.8, 87.7, 91.8 % and *C. papaya*

shows 75.9, 78.7, 82.5, 87.6, 91.5, 96.7% of antioxidant activity (**Table 2 and Figure 1, Figure 2 and Figure 3**). In a DPPH scavenging model, the percentage inhibition of methanolic leaf extracts of *T. ammi*, *M. paniculate*, and *C. papaya* at different concentrations (g/ml) revealed that the antioxidant activity of *T. ammi*, *M. paniculate*, and *C. papaya* increases linearly with increasing concentrations. Similarly, the percentage inhibition of antioxidant activity increased linearly with increasing ascorbic acid concentrations. The high levels of flavonoids and phenol content in the *T. ammi*, *M. paniculate*, and *C. papaya* plants correspond to the antioxidant levels of these plants. DPPH free radical scavenging assay is one of the most frequently used assays as it is advantageous compared to other laboratory-generated free radicals and is accurate. *T. ammi*, *M. paniculate*, and *C. papaya* has strong demonstrable antioxidant activity, according to this report. Free radicals have been linked to the development of a variety of diseases, including atherosclerosis, ischemic heart disease, and cancer, as well as Alzheimer's and Parkinson's disease. Antioxidants can protect the body from free radicals by scavenging or converting reactive metabolites to less reactive molecules.

For all of the plant extracts and standard ascorbic acid, the EC_{50} (concentration needed to achieve a 50% antioxidant effect) was determined. The EC_{50} value is the concentration of extract (antioxidant sample) that can scavenge 50% of the free radical DPPH, as determined by reducing DPPH absorbance after adding extract. The EC_{50} of DPPH can be determined using the regression linear equation of each extract's calibration curve. All of the programs we looked at in this study include EC_{50} values through a mathematical calculation that generates a curve that fits the experimental data points as closely as possible using an effective equation model. To achieve more reliable goodness of fit to the data points, the algorithm changes the parameters by decreasing the sum-of-squares.

EC_{50} of DPPH scavenging activities of *T. ammi*, *M. paniculate* and *C. papaya* shown in **Figure 1**, **Figure 2** and **Figure 3**.

The EC_{50} of methanolic extracts' DPPH scavenging activities was compared to the EC_{50} of the ascorbic acid norm. The EC_{50} value with the lowest value indicates the highest antioxidant activity. EC_{50} less than 50 $\mu\text{g/mL}$ was a very strong antioxidant, 50-100 $\mu\text{g/mL}$ was a strong antioxidant, 101-150 $\mu\text{g/mL}$ was a medium antioxidant, and $EC_{50} > 150 \mu\text{g/mL}$ was a weak antioxidant [18]. The lowest EC_{50} of DPPH scavenging activity indicated the highest antioxidant activity. The methanolic extracts of *T. ammi*, *M. paniculate* and *C. papaya* shows EC_{50} of 47 ± 2 , 46 ± 4 , $45 \pm 3 \mu\text{g/mL}$ respectively. The standard ascorbic acid shows EC_{50} of $43 \pm 1 \mu\text{g/mL}$. These results revealed that the methanolic extracts of *T. ammi*, *M. paniculate* and *C. papaya* strong antioxidant activity when compared to the standard ascorbic acid.

Table 1: The optical density value of the methanolic leaves extracts of *T. ammi*, *M. paniculate* and *C. papaya*

Conc. $\mu\text{g/mL}$	<i>M. paniculate</i>	<i>T. ammi</i>	<i>C. papaya</i>	Standard
	Optical Density (OD) at 517			
Blank	0.5238	0.5238	0.5238	0.5238
75	0.1471	0.1741	0.126	0.0842
100	0.1293	0.1538	0.1112	0.0627
250	0.1052	0.1302	0.0914	0.0394
500	0.0848	0.0852	0.0648	0.0184
750	0.064	0.0514	0.0441	0.0102
1000	0.0426	0.0276	0.017	0.0071

Table 2: Inhibition efficiency of methanolic leaves extracts of *T. ammi*, *M. paniculate* and *C. papaya*

Conc. µg/mL	<i>M. paniculate</i>	<i>T. ammi</i>	<i>C. papaya</i>	Standard
	% of Inhibition			
75	71.917	66.762	75.945	83.925
100	75.315	70.638	78.771	88.030
250	79.916	75.143	82.551	92.478
500	83.811	83.734	87.629	96.487
750	87.782	90.187	91.581	98.053
1000	91.867	94.731	96.754	98.645

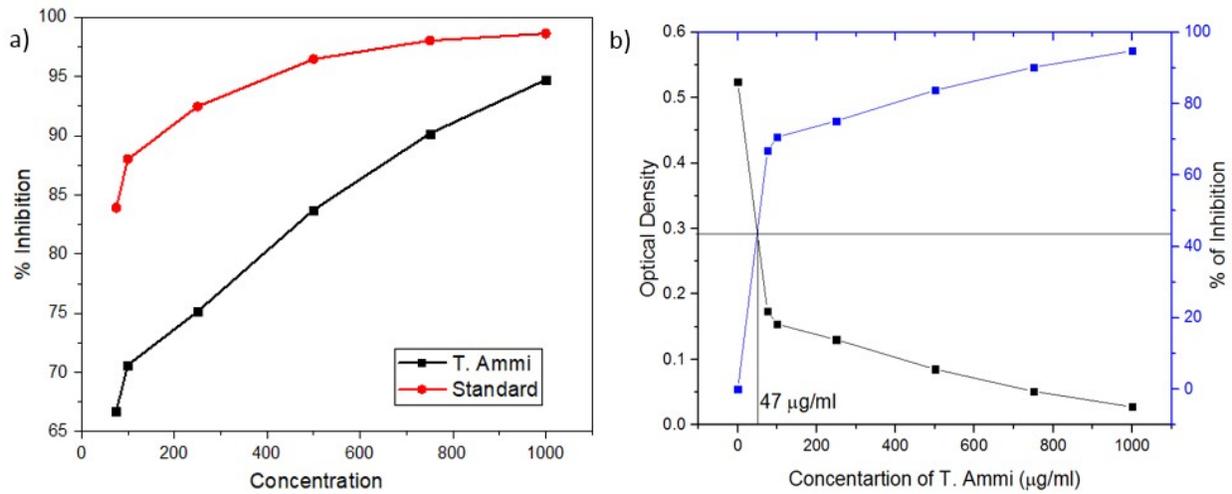


Figure 1: Antioxidant activity (Inhibition efficiency) and EC₅₀ of the methanolic extracts of *T. ammi* DF

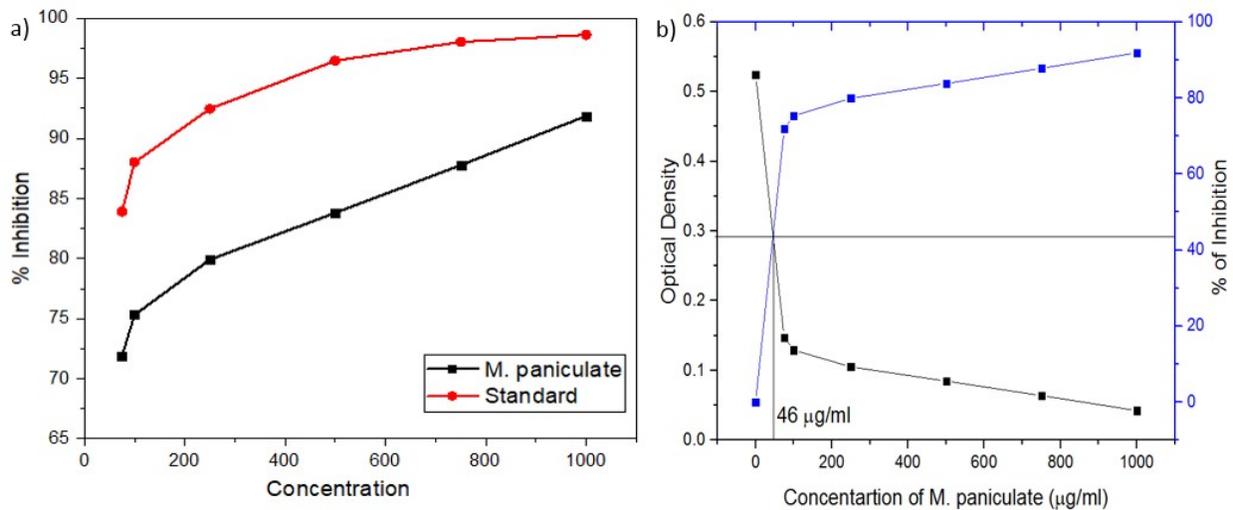


Figure 2: Antioxidant activity (Inhibition efficiency) and EC₅₀ of the methanolic extracts of *M. paniculate*

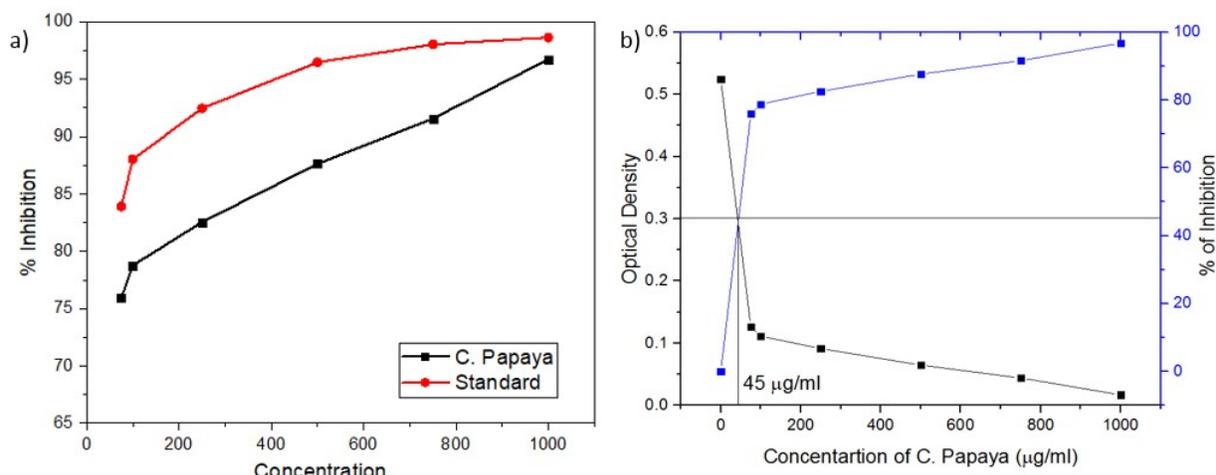


Figure 3: Antioxidant activity (Inhibition efficiency) and EC_{50} of the methanolic extracts of *C. papaya*

CONCLUSION

The DPPH assay method was used to assess the antioxidant efficacy of methanolic extracts of *T. ammi*, *M. paniculate*, and *C. papaya* leaves. This research also discovered that the methanolic leaf extracts of *T. ammi*, *M. paniculate*, and *C. papaya* have major antioxidant properties, making them a readily accessible source of natural antioxidants for everyday usage. The antioxidant properties may be due to a combination of bioactive components. More research is needed to precisely classify, isolate, and probably quantify the components that are responsible for antioxidant activity. The leaf extract appears to be a promising candidate for use as a natural product-based antioxidant for human health.

Conflicts of Interest

All the authors declare no conflicts of interest regarding this manuscript

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