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IN VITRO ANTIOXIDANT PROPERTIES, PHENOL AND FLAVONOID CONTENT OF *MEMECYLON UMBELLATUM*.

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ABSTRACT

The aim of this study was to examine the antioxidant activity, total phenol and flavonoid content of the leaf of *Memecylon umbellatum* collected from four geographically distant regions of Tamil Nadu were examined using extracts of aqueous, ethanol, acetone, chloroform and petroleum ether. The leaf extracts were evaluated for antioxidant activities by DPPH (1,1 – diphenyl -2- picryl-hydrazyl) radical scavenging assay. Among four accessions with different solvents used, maximum antioxidant activity was found in ethanolic leaf extract (84.5%) from Cuddalore followed by others. Total phenol and flavonoid content was quantitatively estimated, the ethanolic leaf extract of *Memecylon umbellatum* was found maximum in Cuddalore accession (31.42 mg Gallic Acid Equivalents (GAE)/g and 12. 21 mg Quercetin Equivalents (QE) /g). The powerful antioxidant activity is attributed to the greater amount of total phenol and flavonoid compound in the ethanolic leaf extract of *Memecylon umbellatum.*

KEYWORDS: *Memecylon umbellatum*, antioxidant activity, DPPH, phenol and flavonoid.

INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Medicinal plants continue to be an important therapeutic aid for

alleviating the ailments of humankind.[1] Therapeutic benefits can be traced to specific plant compounds; many herbs contain dozens of active constituents that together combine to give the plant its therapeutic value.[2] Phytochemical screening of various plants has been reported by many workers.[3],[4] These studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites.[5] A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavengers,[6],[7]

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA.[8],[9] It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others.[10],[11] The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders.[12],[13]

In addition, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. [14] The crude extracts of herbs, spices and other plant materials, rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.[15]

Memecylon umbellatum (Melastomataceae) is an erect tree, herb or shrub, sometimes climbers commonly known as 'Kasa' distributed throughout western peninsular, the eastern and southern parts of India and in the Andaman islands.[16] The leaf powder has antidiabetic potential and also used to treat eye troubles, gonorrhea, leucorrhea, wounds, skin diseases and antioxidant property.[17],[18] Plant contains a wide variety of phytoconstituents such as umbellactone, β-amyrin, Oleanolic acid, ursolic acid, sitosterol and organic acids. Hence in the present study, the leaf extract of *Memecylon umbellatum* was screened for Phenolic and

antioxidant properties. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants.

MATERIALS AND METHODS

Collection of *Memecylon umbellatum*

The healthy plants of *Memecylon umbellatum* were collected from four different regions of Tamil Nadu namely Vizhupuram, Cuddalore, Potheri and Uthukottai. The collected plants were brought to the laboratory and maintained at Poonga Biotech Research Centre, Plant biotechnology division, Chennai - 600 094, Tamil Nadu, India.

Preparation of the plant extract

Preparation of the extracts was done according to a combination of the methods used by Pizzale *et al.*,[19] and Lu and Foo.[20] About 15g of dried leaf fine powder of *Memecylon umbellatum* plant materials were extracted with 15 ml ethanol (75%), acetone, chloroform, petroleum ether and aqueous extract for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10ºC.

Qualitative analysis of Antioxidant activity of *Memecylon umbellatum*

The antioxidant activity of leaf extracts of *Memecylon umbellatum* was determined by following the method as described by Selvaraj *et al.*[21]

50µl of leaf extracts of *Memecylon umbellatum* were taken in the microtiter plate. 100µl of 0.1% methanolic DPPH (1,1-Diphenyl-2-picryl hydrazyl) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of Free radical scavenging activity of *M. umbellatum*

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Leaf extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially,

absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control.[22] Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula.

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100

Estimation of Total phenol content in *Memecylon umbellatum*

Total phenolic content in the ethanolic leaf extracts was determined by the Folin Ciocalteau colorimetric method.[23] For the analysis, 0.5 ml of dry powdered ethanolic leaf extracts were added to 0.1 ml of Folin-Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (Na_2CO_3) was added and the mixture was allowed to stand for 30 min after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in *Memecylon umbellatum*

Total flavonoids content in the ethanolic leaf extracts was determined by the aluminium chloride colorimetric method.[24] 0.5 ml of leaf extracts of *Memecylon umbellatum* at a concentration of 1mg/ ml were taken and the volume was made up to 3ml with ethanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

RESULTS AND DISCUSSION

Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources. Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or

mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants.[25],[26]

Wild accessions of *Memecylon umbellatum* leaf samples were used for antioxidant studies. Analysis on different extraction of ethanol (75%), acetone, petroleum ether, chloroform and aqueous extract showed the presence of antioxidants. 50µl of leaf extracts (acetone, ethanol, petroleum ether, chloroform and aqueous extracts of *Memecylon umbellatum* were estimated for free radical scavenging activity using Diphenyl-2-picryl hydrazyl (DPPH) assay. The samples observed for its bleaching from purple to yellow and pale pink were considered as strong positive and weak positive respectively (Table (1). Among the four wild accessions and five different solvent extracts of *Memecylon umbellatum*, the ethanolic leaf extract collected from Cuddalore accession recorded the most effective DPPH radical scavenging activity (84.5%) followed by Vizhupuram (73.9%), Potheri (69.6%) and Uthukottai (64.8%) accessions Fig.(1), (2). Cuddalore accession values being close to synthetic antioxidant (BHT) as positive control (98.4%). In each case, ethanolic leaf extracts recorded higher percentage of free radical scavenging activity than acetone extractions followed by aqueous, petroleum ether and chloroform extract.

Phenolics are the most widespread secondary metabolite in plant kingdom. Phenolic and flavonoid compounds are a class of antioxidant agents which act as free radical terminators. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable.[27],[28] In our study, total phenol and flavonoid content of *Memecylon umbellatum* leaf extract was estimated by using Folin-Ciocalteau colorimetric method and aluminium chloride method. The results of the present study showed that the phenol and flavonoid contents of the ethanolic leaf extracts in terms of Gallic Acid Equivalents (mgGAE)/g) and Quercetin equivalent (mg QE/g) were found to be maximum in *Memecylon umbellatum* (Cuddalore) (31.42 mg Gallic Acid Equivalents (GAE)/g and 12.21 mg Quercetin Equivalents (QE) /g) Table (2). It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers[6] (Evans *et al.*, 1995). Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds. [30], [31]

In conclusion, antioxidant activity, total phenol and total flavonoid content of medicinal plants is very important in identifying new sources of therapeutically and industrially

important compounds. It is imperative to initiate an urgent steps for screening of plants for secondary metabolites. The present research work attempts to assess the importance of antioxidant activity, total phenol and total flavonoid properties in leaves of *Memecylon umbellatum* to improve the health status of people and also to use in nutraceutical products of commercial importance. The results indicate that the plant material may become an important source of compounds with health protective potential.

Mother plants of *Memecylon umbellatum* (Cuddalore)

Table (1): Qualitative analysis of antioxidant activity from leaf extract of *Memecylon umbellatum*.

S.No	Extractions	*Memecylon umbellatum* - Leaf			
		Cuddalore	**Vizhupuram**	**Potheri**	**Uthukottai**
	BHT (standard)	++	++	++	++
S1	Aqueous	++	+	+	+
S2	Acetone	+	+	+	+
S3	Ethanol	++	+	+	+
S4	Chloroform	+	Semi positive	Semi positive	Semi positive
S5	Petroleum ether	+	Semi positive	Semi positive	Semi positive

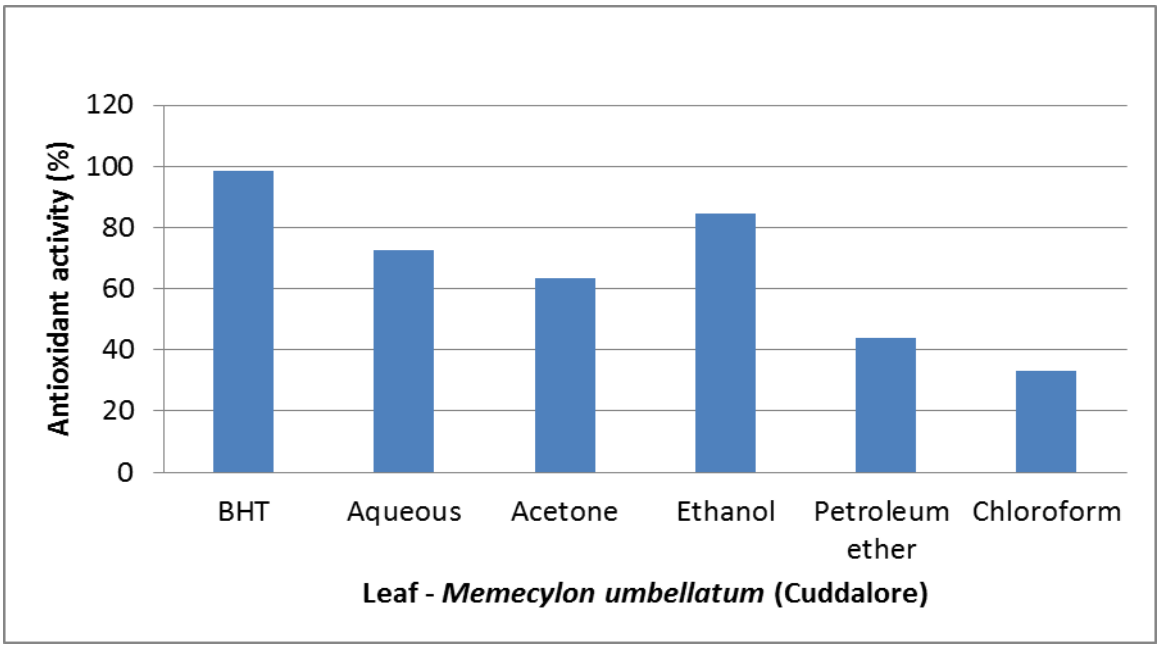

Figure 1. Antioxidant activity from leaf extract of *Memecylon umbellatum* (Cuddalore)**.**

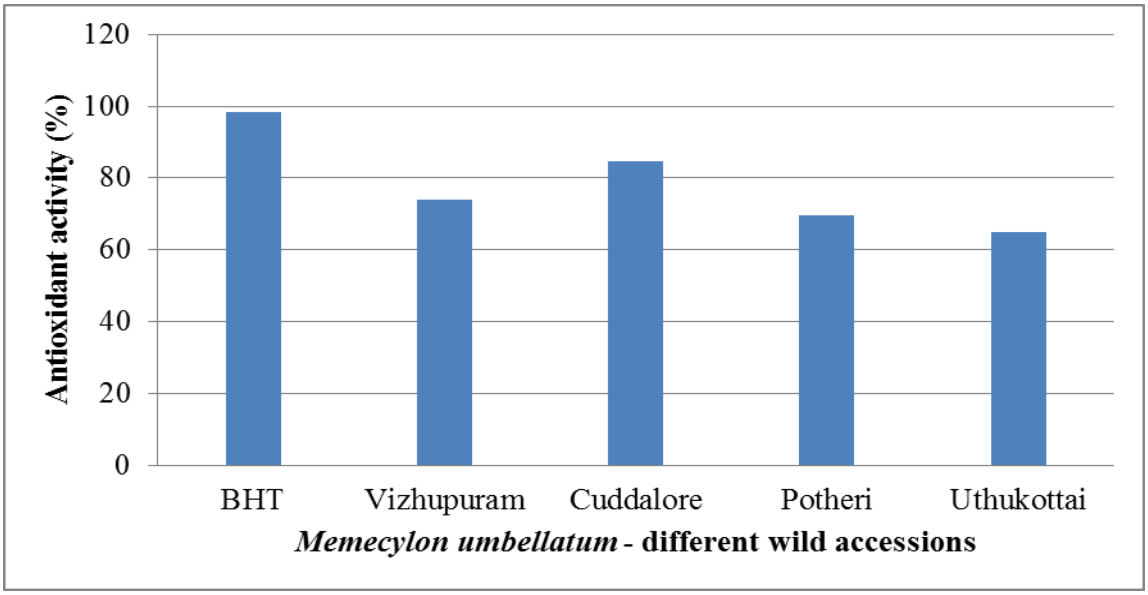

Figure 2. Antioxidant activity from ethanolic leaf extract of *Memecylon umbellatum* different wild accessions.

Table (2): Estimation of Total phenol content from ethanolic leaf extract of *Memecylon umbellatum*.

Test	Leaf of *Memecylon umbellatum* – Different accessions			
	Cuddalore	Vizhupuram	Potheri	Uthukottai
Total phenol content (mg GAE/g)	31.42	26.35	23.17	12.24
Total flavonoid content (mg QE/g)	12.21	10.9	8.78	5.87

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