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Antioxidant potential and antimicrobial activity of *Cinnamomum malabathrum* (Batka)

B. HARI KUMAR[1], SHANI BASHEER[1] and HASEENA[2]

[1]Department of Chemistry, T K M College of Arts and Science, Kollam - 691 005 (India).
[2]CEPC Laboratory, Kollam - 691 001 (India).

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ABSTRACT

Cinnamomum malabathrum (Batka) is an aromatic tree which is locally known in Malayalam as Vayana. They are very popular species commonly used in Indian dietary. The antioxidant activity of essential oil was evaluated by using DPPH. The phytochemical investigation indicates the presence of phenolics and flavanoids. The aqueous extract was also studied. The results were found to be significant when compared to standards. The antimicrobial activity of the essential oil was found to be insignificant.

Key words: *Cinnamomum malabathrum*, DPPH, Phenolics, Flavanoids, antimicrobial, essential oils.

INTRODUCTION

Free radicals and other reactive oxygen species, collectively known as ROS are generated continuously via normal physiological processes, more so in pathological conditions[1]. The role of free radicals and active oxygen in the pathogenesis of human diseases including cancer, ageing and atherosclerosis has been recognised[2]. Antioxidant enzyme as well as some other natural antioxidants such as reduced glutathione, phenolics, flavanoids, pigments, constitute a system that keeps ROS at a low steady state concentration in cells and tissues and prevent oxidative situations.

Cinnamomum malabathrum (Batka) belonging to the family Lauraceae comprises about 250 species which are distributed in India, China, Srilanka and Australia. Cinnamomum leaves are used extensively as spices in food or to produce essential oils. The plant leaf has a hot taste and emits a spicy odor when crushed[3]. Previous studies on biochemical activities from Cinnamomum were mainly focused on its essential oils[4] which included antioxidant, antimicrobial activity[5] and antidiarrhoeal activity[6]. Cinnamomum leaf used as a food and also folk medicine in India and China for the treatment of inflammation, gastritis, blood circulation, liver and spleen disorders but there is limited investigation concerning the use of Cinnamomum leaf as a natural antioxidant[7]. Unfortunately, little information on a comparison of antioxidant activity between various species of Cinnamomum leaves has been available.

MATERIAL AND METHODS

General

The chemicals 1,1-diphenyl -2-picryl hydrazyl(DPPH) was purchased from Sigma Aldrich. Gallic acid, Quercetin, BHA, BHT and α-tocopherol were purchased from Merck India, Mumbai. All other chemicals used were of analytical reagent grade.

Plant material

The fresh leaves were collected from Kollam area of Kollam district in January 2010. The plant was identified by Dr. N. Mohan, Scientist, TGBRI, Palode.

Isolation of essential oil

The plant leaves were cut into small pieces and shade dried. The essential oils were obtained by two different methods. Fresh leaves (1375 g) were subjected to hydro distillation using a Clevenger type apparatus for 8 hours. To obtain essential oil by steam distillation (980 g) material were subjected to steam for 6 hours and then the volatile components in the steam were isolated by extraction with ether. The oils were subsequently dried over anhydrous sodium sulphate and stored in sealed vials until used. The essential oil isolated by steam distillation and hydro distillation from these sample gave 0.000840 g (w/w) and 0.000446 g (w/w) respectively. The essential oil obtained by hydro distillation was kept aside for comparing the chemical constitution.

Preparation of crude extract of *Cinnamomum malabathrum*

The dried plant material (50 gm) was grinded and then extracted with distilled water. The supernatant were evaporated and dried to get 1.4178 gm (0.028356w/w).

DPPH radical scavenging activity

The antioxidant activities of the extract and oil based on the scavenging activity of the stable 1,1-diphenyl-2 picryl hydrazyl (DPPH) free radical was determined by the method described by Braca. *et.al.,*[8]. The test samples 1 ml to 4 ml were taken and 1ml of 0.008% methanol solution of DPPH were added and made up to 5 ml with alcohol or distilled water. A blank was also prepared. After 30 minutes absorbance at 517 nm was determined and the percentage inhibition activity was calculated as:

$$\text{Percentage inhibition activity} = \{[A_0-A_1]/A_0\} \times 100$$

where A_0 was the absorbance without sample and A_1 was the absorbance with sample. α-tocopherol, BHA, and BHT were used as standards (Table 1,2).

Determination of total phenolic content

The modified Folin Ciocalteu method was used for the determination of total phenolic content in the given extract [9,10]. An aliquot of the extract were mixed with 0.5 ml Folin Ciocalteu reagent and allowed to stand for 3 minutes followed by the addition of 2 ml of sodium carbonate. After one hour of standing the absorbance was measured at 630 nm. Total phenolic content was expressed as mg/g gallic acid equivalent and calculated as

$$C = c.v/m$$

where C = total phenolic content in mg/g plant extract, c = concentration of gallic acid established via calibration curve, v = volume of plant extract, m = weight of plant extract in gm (Table 3).

Determination of total flavanoids

The method used for determination of total flavanoids content was modified by Jia.et.al. The standard used for the analysis was quercetin. Five concentration (1, 5, 10, 15, 25 & 50 µg/ml) were prepared by dissolving quercetin standards in deionised water or ethanol. 2 ml from each concentration was taken and labelled as S_1 to S_5. A blank was also prepared. 0.3 ml of 5% sodium nitrate solution was prepared and allowed to stand for 5 minutes followed by the addition of 0.6 ml 10% $AlCl_3$ solution. It was kept for 5 minutes and then mixed with 2 ml of 1 M NaOH solution. The absorbance of the final solution was measured at 510 nm. Total flavanoids were expressed as mg/g quercetin equivalent and calculated as:

$$C = c.v/m$$

where C = total flavanoids content mg/g plant extract, c = concentration of quercetin established via calibration curve, v = volume of extract , m = weight of plant extract in gm (Table 3).

Antimicrobial activity

Bacterial species representing gram positive and gram negative strains for the testing purpose were obtained from MTCC, Institute of Microbial Techonology, Chandigarh, India. Microbial activity was evaluated by using a filter paper disc diffusion method. The degree of growth inhibition was evaluated after the incubation period of 24 hours at 37^0C. Three replicates of each were performed and the mean value was recorded.

RESULTS AND DISCUSSION

DPPH free radical activity

The DPPH radical is widely used as a

model to investigate the free radical scavenging of several plant extract. *Cinnamomum malabathrum* extract scavenged the DPPH radical in a concentration dependent manner. IC_{50} for the aqueous extract for *Cinnamomum malabathrum* was 770 µg/ml and for the essential oil is 1700 ìg/ml. The standards BHA, BHT and ∞-tocopherol showed IC_{50} = 840, 900, & 840 µg/ml respectively. This shows that the free radical scavenging activity of aqueous extract is much higher than the standard compound whereas the scavenging activity of essential oil is very less.

Total phenolic and flavanoid content

The major plant compounds, polyphenolics played an important role in absorbing and neutralising free radicals, quenching singlets and triplet oxygen and decomposing peroxides. The results from the study strongly suggest that the phenolics are important component of these plants and some of their pharmacological effects may be due to the presence of phenolics. The total phenolic content in mg/g gallic acid equivalent was found to be 0.0324 mg/g in aqueous extract of

Table 1: Antiradical activity of aqueous extract of *Cinnamomum malabathrum* observed with DPPH

Samples	Concentration (µg/ml)	%Inhibition	IC_{50} (µg/ml)
Aqueous extract	100.74	6.9956	770
	201.48	14.0537	
	302.22	18.5509	
	402.96	27.2954	

Table 2: Antiradical activity of essential oil of *Cinnamomum malabathrum* observed with DPPH

Samples	Concentration (µg/ml)	%Inhibition	IC_{50} (µg/ml)
Essential oil	192.96	-	1700
	385.92	1.4972	
	578.88	3.558	
	771.84	7.112	
BHA			840
BHT			900
α-tocopherol			840

Table 3: Total phenolics and flavanoids content of *Cinnamomum malabathrum*

Sample	concentration (mg/g) equivalent
Aqueous extract	0.0324 mg/g gallic acid equivalent
Aqueous extract	0.0825 mg/g quercetin equivalent

Table 4: Antimicrobial activity of the essential oil of *Cinnamomum malabathrum*

Microorganisms	Growth inhibition (mm) due to essential oils and their different dilutions [a,b]							
	1:0	1:1	1:2	1:4	1:6	1:8	1:10	1:12
Bacteria								
Staphylococcus aureus MTTC -96	21	12	14	7	-	-	-	-
Escherichia coli MTTC -118	36	10	11	8	6	-	-	-
Salmonella typhi MTTC -733	22	16	11	6	-	-	-	-
Pseudomonas aeruginosa MTTC -424	12	10	10	6	-	-	-	-
Bacillus coreus MTTC -430	18	11	12	11	7	-	-	-
Fungii								
Saccharomyces cerevisiae MTTC -36	16	N D						
Aspergillus flavus MTTC -277	15	N D						
Candida utilis MTTC -183	6	N D						

[a]Including diameter of the filter paper disk.

[b]Mean value of three independent experiments.

N D- Not determined

Cinnamomum malabathrum. The total flavanoids in mg/g quercetin equivalent were found to be 0.0825 mg/g in aqueous extract of *Cinnamomum malabathrum.*

Antimicrobial activity

The *Cinnamomum malabathrum* essential oil showed considerable microbial activity. The antibacterial and antifungal activities result seems to be in accordance with previous results, indicating that the essential oil possess high levels of antimicrobial activity (Table 4). The antimicrobial activity clearly shows the inhibitory activity of the essential oil against microorganisms. The available literature shows the essential oils are used in food technology[11], and pharmaceutical industry[12].

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