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Determination of Total Flavonoid, Antioxidant, Antidiabetic, and Antibacterial Activities of Flavonoids Present In *Memecylon malabaricum* (C.B.Clarke) Cogn

Research Article

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Abstract

Flavonoids are the secondary metabolites that are found in the plants which possess the various biological properties. The present study deals with the total flavonoid content, antidiabetic, antioxidant and antibacterial activities of Leaf flavonoid fraction (LFF) isolated from *M. malabaricum.* Flavonoid were isolated and quantified as previously described. Antidiabetic, antioxidant and antibacterial activities were evaluated for Leaf flavonoid fraction (LFF) of *M. malabaricum* leaves. The Total Flavonoid content was found to be 553.5865±18.56 mg CE/g. IC50 of LFF for α-amylase enzyme and α-glucosidase enzyme 183.95 and 37.720 µg/ml respectively. At 100 µg/ml concentration, the scavenging activity of LFF reached around 54.46% for DPPH and 58.32% for Nitric oxide. While the LFF has significantly inhibited both gram-negative *Pseudomonas aeruginosa* and gram-positive *Staphylococcus aureus* bacteria by showing the maximum zine of inhibition of 8.33 ± 1.15 and 9.66 ± 0.57 respectively. These results exhibited that the flavonoids in LFF have the potential Antidiabetic, antioxidant and antibacterial properties.

Keywords: *M. malabaricum*; Flavonoid Content; Antidiabetic Activity; Antioxidant Activity; Antibacterial Activity.

Introduction

Flavonoids are a broad class of polyphenolic chemicals found in plants and other natural sources. They are divided into six groups: flavanones, flavones, isoflavones, flavonols, flavanols, and anthocyanins. These compounds have sparked widespread interest due to their diverse biological actions, which include anti-inflammatory, antioxidant, antiviral, antibacterial, anticancer, cardioprotective, and neuro protective properties[1-5]. Flavonoids have been investigated for their possible health advantages, and their consumption has been linked to a variety of positive impacts on human health, making them appealing to the medical, pharmaceutical, cosmetic, food, and nutraceutical industries [6]. Melastomataceae is a dicotyledonous flowering plant family that is primarily found in the tropics. There are approximately 175 genera and 5115 recognized species. Annual or perennial plants, shrubs, and small trees are all members of this family [7].

Memecylon malabaricum is a plant in the family Melastomataceae. It is indigenous to India and is found in the Western Ghats region. The plant is well-known for its traditional usage in the treatment of diabetes, numerous bacterial infections, inflammatory and skin illnesses such as herpes, and chickenpox. The plant's leaves were tested using GC-MS, and the results revealed the presence of a variety

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of bioactive chemicals [8]. Several research have been conducted on the plant, including antihelmintic activity, anti-psoriatic, anticancer, and antibacterial activities for the crude extract [9-12]. The crude methanolic extract was tested for Antidiabetic efficacy *in vivo* [13]. Since the crude methanolic extract may contain numerous metabolites in it, purification of each compound is needed. For the current investigation, we had treated the methanolic extract further with Amberlite XAD4 for the elution of flavonoids. The current study focuses on isolating flavonoids from a methanolic extract of *Memecylon malabaricum* leaves and evaluating their in vitro antidiabetic, antioxidant, and antibacterial effects.

Materials and methods

Collection and preparation of plant extract

M. malabaricum leaves were collected in Shivamogga, Karnataka, India, specifically in Chakranagar, Hosanagar Taluk. After washing the fresh leaves in running tap water to remove any surface impurities, they were rinsed with distilled water and dried in the shade for 15 days, or until their dry weight remained steady. The dried leaves were mashed using an electric blender to extract the most phytochemicals by reducing particle size. After that, the ground leaves were sealed in an airtight container. Using a Soxhlet apparatus, the powdered leaves were defatted with n-Hexane, and flavonoids were isolated using conventional procedures. Every solvent used for the extraction process was carried out for roughly 24 hours. After concentrating through a rotary evaporator, the resultant extract was kept refrigerated until it was processed further.

Isolation of Flavonoids

The methanolic extract was partitioned against diethyl ether to eliminate all non-polar components. Furthermore, non-aromatic components other than flavonoids, such as free sugars and aliphatic acids, were extracted from the extract via adsorption chromatography with Amberlite XAD-4, which adsorbs polyphenolic chemicals, including flavonoids. This was done according to [14] Dong et al., (2014). The adsorbed polyphenolic chemicals were eluted with 100 percent methanol. Which was then dried at 37 ^{0}C and stored at 4 ^{0}C until further use. The Leaf Flavonoid fraction (LFF) was separated and collected as outlined by Dong et.al.

Quantification of Flavonoids

Aluminium chloride method was used with slight modification to determine the total flavonoid content present in LFF [15]. The absorbance of the reaction mixture was determined at 415 nm using a PC-based UV-visible spectrophotometer (Systronics, Model-119). Catechin was used to create the standard curve (20-100 µg/mL), and the results were expressed as µg of Catechin equivalents per mg of extract.

FTIR analysis of LFF

FTIR spectroscopy was utilized to determine the type of functional groups contained in the LFF. The LFF of *M. malabaricum* was dissolved in methanol (1 mg/ml). The sample was evaluated using Bruker FTIR spectroscopy (USIC, Davangere University, Davangere, Karnataka, India) between 400 and 4000 cm^{-1}. Infrared spectrum data were collected to detect functional groups, as stated by Silverstein [16].

Anti-diabetic activities

LFF were examined for α-amylase inhibition with the 3,5-dinitrosalicylic acid method [17]. LFF's capacity to inhibit α-glucosidase was evaluated using p-nitrophenyl-α-D glucopyranoside as a substrate [18]. Acarbose served as the positive control for the both enzyme inhibition activity. The inhibitory action was defined as the half maximum inhibitory concentration (IC50), which measures the extract's ability to suppress enzyme activity.

Inhibitory activity (%) = (1 − As/Ac) ×100

Where, As= absorbance of sample, Ac = absorbance of control

Antioxidant activities

The 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method is widely used to assess the free radical scavenging ability of natural substances [19]. *M. malabaricum*'s LFF free radical scavenging activity was assessed in comparison to DPPH. Nitric oxide radical scavenging was also employed to determine the LFF's antioxidant capacity [20] . Ascorbic acid has served as the benchmark for both radical scavenging activities.

% Inhibition= Absorbance of control-Absorbance of sample X 100/Absorbance of control

Antibacterial activity

The agar-well diffusion technique [21] was used to assess LFF's antibacterial activity following minor alterations. The samples were analyzed for gram-positive *Staphylococcus aureus* (MTCC-7443) and gram-negative *Pseudomonas aeruginosa* (MTCC-1034) bacteria. The inoculum was adjusted to about 5×10^5 CFU/ml using sterile saline solution. Samples were dissolved in DMSO at a concentration of 10 mg/ml to create a stock. Concentrations ranging from 200-800 µg/ml were separated into distinct wells. Every antibacterial activity was performed using Mueller Hinton agar medium [38]. Microorganisms were cultivated at 37 ^{0}C, and the inhibitory zone diameter (in millimeters, mm) was determined.

Determination of Minimum Inhibitory Concentration (MIC): The minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth dilution technique [22]. Samples were diluted in DMSO from 0 to 800 µg/ml (w/v) of stock solutions. The samples were examined for gram-negative *Pseudomonas aeruginosa* (MTCC-1034) and gram-positive *Staphylococcus aureus* (MTCC-7443). Using a micro-pipette, add 10 µl of inoculum adjusted to 5 x 10^5 CFU/ml to the appropriate wells and mix with the growth medium. Bacterial plates were incubated for 48 hours at 37 ^{0}C. The MIC values show the lowest quantity that inhibits the growth of the tested microorganisms. After incubation, 50 µl of test-culture from the obviously inhibited well was put on an agar well plate and incubated. The concentration with no growing colony was determined MBC.

Results and discussion

Total flavonoid analysis

Total flavonoid content was quantified using aluminum chloride method. Numerous important phytochemicals have shown their

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strong presence that has proved the way to estimate the flavonoid content in *M. malabaricum* leaf flavonoid fraction. The yield of flavonoid rich *M. malabaricum* leaf flavonoid fraction recorded as 553.5865±18.56 mg of catechin equivalents per mg of LFF (Y = 0.0032x + 0.0289, R2 = 0.9969; Y = 1.8373). The linear regression of leaf flavonoid fraction represented in **(Figure 1)**.

Fourier transforms infrared spectrometer (FTIR)

Infrared spectrum can be obtained due to the molecular vibration by the solid, liquid, or gas samples through absorbing or emitting of infrared ray. The method of obtaining the spectrum is known as Fourier-transform infrared spectroscopy (FTIR) [23]. The FTIR spectra of flavonoids have been extensively studied, and the flavonoid structure shares certain wavenumber ranges [24]. The results obtained from the FTIR spectra of *M. malabaricum* LFF (Figure.2) confirmed the existence of several functional groups associated to flavonoids, which are listed in **Table 1**.

The FTIR Spectrum of flavonoids exhibits the carbonyl frequency at 1642cm^{-1}. The substituents such as hydroxyl/alkyl at 3 or 5 position do not markedly affect the carbonyl frequency. IR spectra of leaf flavonoid fraction showed frequencies at 3329 cm^{-1} and at 2944–2833 cm^{-1} indicating the presence of hydroxyl group and C–H in conjugation, respectively and the absorption peaks at 1642, and 1442 cm^{-1} indicated the presence of C=O group and unsymmetric ethylenic double bond and aromatic rings [25,26]. Presence of -OH, and -C=O absorption bands are characteristics of flavonoids.

Antidiabetic activities

Enzymes like α-amylase and α-glucosidase hydrolyze starch into maltose and glucose. Although different prescribed anti-diabetic medicines can help treat diabetes mellitus, they have a number of negative effects. A naturally occurring selective inhibitor for α-amylase and α-glucosidase activity is necessary [27]. The preliminary study on the inhibition of α-Amylase in vitro [28] and in vivo (Ramaiah M et.al., 2012) in dose dependent manner for crude methanolic extract of *M. malabaricum* plant. In the present study, LFF inhibited α-amylase by 28.45% (IC50 183.95 µg/ml), while regular Acarbose inhibited it by 83.53% (IC50 57.31 µg/ml). LFF inhibited α-Glucosidase at a maximum of 65.94% (IC50 37.720 µg/ml), while conventional Acarbose inhibited α-Glucosidase at 91.69% (IC50 19.379 µg/ml). The inhibition of α-amylase and α-glucosidase enzymes against LFF and the standard acarbose is depicted in (Figure 3) and (Figure 4). The present study revealed that LFF shown the inhibition of α-amylase and α-glucosidase enzymes in dose-dependent manner.

Antioxidant activities

Antioxidants are chemicals that can quench free radicals and mitigate their deleterious effects. Their functioning is dependent on the donation of an electron, which facilitates the stability of unpaired

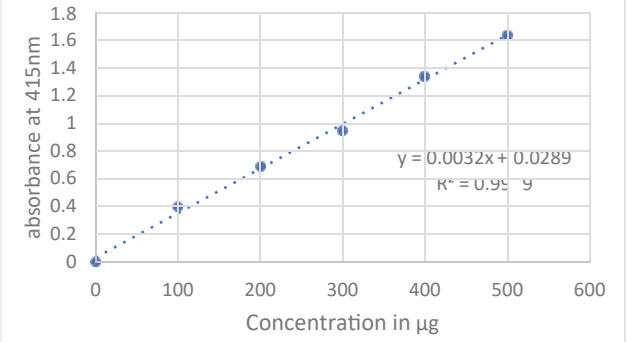

Figure 1: Total flavonoid content using linear regression of M. malabaricum LFF.

Table 1: functional groups identified through FTIR spectra of LFF of *M. malabaricum*.

Wave number	Bonds	Functional group
3329	O-H Stretch	Alcohols and phenols
2944	O-H/C-H stretch	Alcohols/ Aromatics
2833	C-H Stretch	Aromatic hydrocarbons
1642	C=C Stretch	Alkenes
1442	C=O stretch	Carbonyls
1212	C-C-O Stretch	Alkyl or aryl peroxidases
1113	C-O-C Stretch	Aliphatic ethers
1018	C-O-C Stretch	Esters

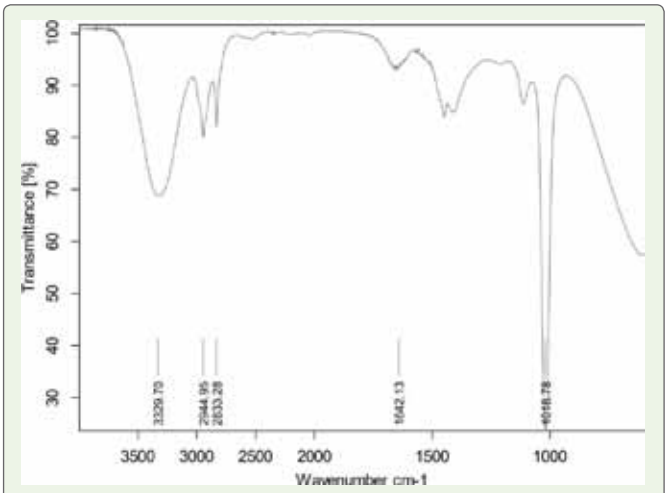

Figure 2: FTIR spectra of LFF.

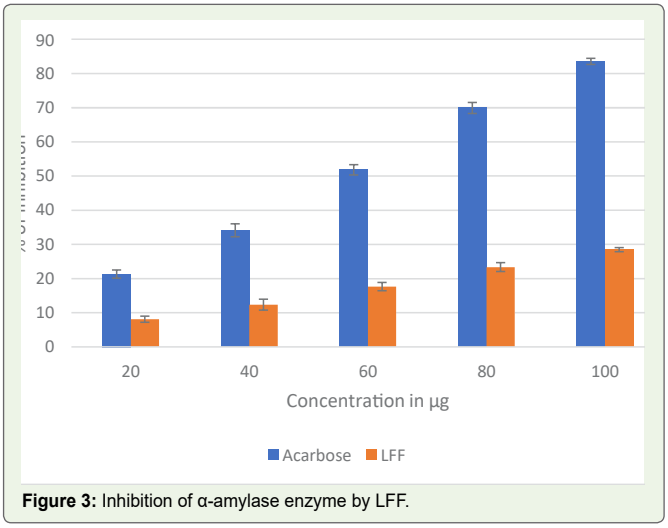

Figure 3: Inhibition of α-amylase enzyme by LFF.

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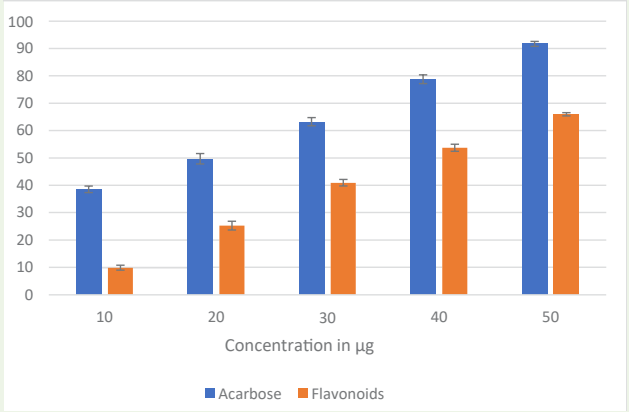

Figure 4: Inhibition of α-glucosidase enzyme by LFF.

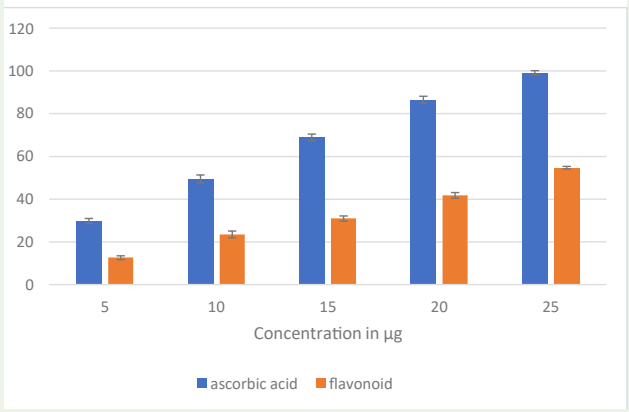

Figure 5: Inhibition of DPPH free radical by LFF.

electrons within free radicals while remaining inert themselves. Scavenging is a procedure that neutralizes free radicals [29]. Previous studies on the antioxidant activity of the *M. malabaricum* plant utilized crude methanolic extracts exhibited the antioxidant properties against DPPH and ABTS radicals [30, 12]. In this study, different concentrations of *M. malabaricum* LFF were tested using the 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and nitric oxide free radical scavenging techniques. The LFF had the highest DPPH activity of 54.46% (IC50 value 23.44 µg/ml), whereas ascorbic acid showed around 96.9% (IC50 value of 10.242 µg/ml) (Figure 7a). The LFF showed the highest nitric oxide radical scavenging activity at 58.32% (IC50 of 89.41 µg/ml), whereas ascorbic acid showed 41.71% (IC50 of 59.439 µg/ml) suppression. These study reports suggested that *Memecylon malabaricum* LFF have shown the significant antioxidant properties by scavenging the DPPH and Nitric oxide radicals.

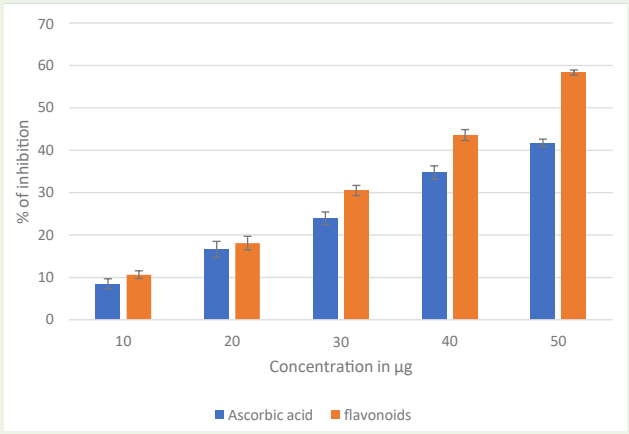

Figure 6: Inhibition of nitric oxide free radical by LFF.

Antibacterial activity

Flavonoids can prevent the growth of many bacteria by interfering with their cell membranes, energy metabolism, nucleic acid production, and virulence factors. Flavonoids can also improve the effectiveness of medications and reverse antibiotic resistance in some bacteria. As a result, flavonoids are promising candidates for creating novel antibacterial agents or adjuvants [31]. The general modes of action of antibacterial agents include inhibiting the synthesis of proteins and nucleic acids, damaging the integrity of cell membrane permeability [32-35].The flavonoids are believed to depend on the interaction with the penetration of hydrophobic core and hydrophilic region of the phospholipids on the cellular membrane [36,37]. Several research studies have sought out elucidate the antibacterial modes of action of selected flavonoids as well. Previous studies found that the *Memecylon malabaricum* leaves crude methanolic extract showed the inhibition against *E. coli, S. aureus, P. aeruginosa, B. subtilis,* and *S. typhi* [9, 12]. The current study employed the agar well diffusion technique to determine LFF's antibacterial effectiveness against both gram-positive and gram-negative bacteria. (Figure7) exhibits the zone of inhibition in agar well plates for the antibacterial efficacy of LFF and the standard Kanamycin. **Table 3** is a compilation of bacteria's inhibitory zones. **Table 4** shows the MIC and MBC for LFF. All the

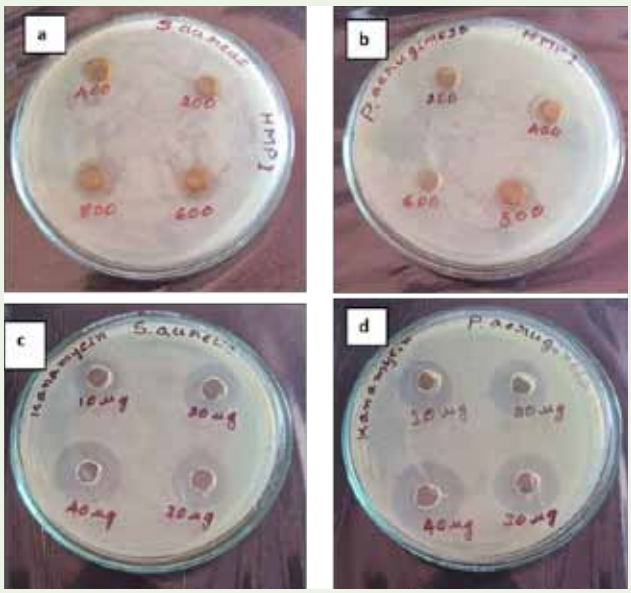

Figure 7: Antibacterial efficacy of LFF and standard kanamycin **a.** LFF against *S. aureus* **b.** LFF against *P. aeruginosa* **c.** kanamycin against *S. aureus* **d.** kanamycin against *P. aeruginosa*..

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Table 2: IC50 values of DPPH and Nitric oxide invitro antioxidant efficacy of LFF of *M.malabaricum leaves*

Sample Name	DPPH (µg/ml)	Nitric oxide (µg/ml)
Ascorbic acid	10.242	59.439
LFF	23.44	89.41

Table 3: Antibacterial properties of samples in inhibition zone (in millimeter, mm)

Sl. No.	Sample Name	Conc. (µg/ml)	*Staphylococcus aureus* (mm)	*Pseudomonas aeruginosa* (mm)
1	LAF	200	---	---
		400	---	---
		600	6.33 ± 0.57	5.66 ± 0.57
		800	9.66 ± 0.57	8.33 ± 1.15
2	Kanamycin	10	8.33 ± 0.57	8.66 ± 0.57
		20	11.66 ± 0.57	11.33 ± 0.57
		30	13.33 ± 1.15	14.66 ± 0.57
		40	17.66 ± 0.57	17.33 ± 0.57

Table 4: Antibacterial properties in MIC and MBC

Sl.	Sample name	Staphylococcus aureus		*Pseudomonas aeruginosa*(µg/ml)	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	LFF	200	600	200	600
2	Kanamycin	5	20	5	20

antibacterial activity was compared to standard Kanamycin. These study reports suggested that *Memecylon malabaricum* leaf flavonoid fraction have shown the significant antibacterial activity against *S. aureus* and *P. aeruginosa.*

Conclusion

Flavonoids are a large family of polyphenols with a wide range of biological properties, making them intriguing to researchers from a number of fields. They have the potential to improve health because of their high antioxidant capacity both *in vivo* and *in vitro* systems. Their widespread existence and diversified biological function make them a promising subject for future research and development. The current work focuses on the anti-diabetic, antioxidant, and antibacterial properties of flavonoids extracted from the plant *M. malabaricum* cogn. The current investigation found that LFF is containing the flavonoids those of enzyme inhibitors for α-amylase and α-glucosidase, as well as free radical scavengers. LFF also exhibited promise inhibition of antimicrobial activity, which MBC subsequently corroborated. However, while flavonoids show promising results *in vitro*, further research is needed to isolate them, understand their structure and mechanism of action, and demonstrate their therapeutic potential *in vivo*.

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