Nutritive Value, Antioxidant and Antimicrobial Activities of Useful Wild Medicinal Plant: *Callicarpa tomentosa* (L.) L.

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Abstract

Callicarpa tomentosa (L.) L. (Family- Lamiaceae) was a widely used wild medicinal plant. In Myanmar, it was known as Kyun nalin and English name is Velvety beauty berry. It was a medium-sized tree reaching a height of up to 5 m and found mainly in India, Sri Lanka, Indonesia, Myanmar, Nepal, and the Malay Peninsula. Various parts of this plant are used in traditional medicine. The present study was the investigations of nutritive value, antioxidant and antimicrobial activities of *Callicarpa tomentosa* leaves. The leaves were collected from Myitkyina University campus during 2021-2023. The nutritive value was determined by AOAC method. According to the results, crude fibre content was found to be highest value (31.67%). Antioxidant activity of leaves was also determined by using DPPH radical scavenging assay. The IC_{50} value of ethanol extract of dried leaves was found to be 105.96 µg/mL. Agar-well diffusion method was used to examine the antimicrobial activity of three extracts on seven tested microorganisms. Ethanol and methanol extracts were found to be the maximum inhibition zone (40 mm) against on *Agrobacterium tumefaciens*.

Keywords: *Callicarpa tomentosa*, Lamiaceae, leaves, nutritional, DPPH, antimicrobial

Introduction

The genus *Callicarpa* (Family-Lamiaceae) comprises about 140 species of small trees and shrubs distributed in tropical and subtropical locations. In Greek, the genus name *Callicarpa* means callos, “beauty” and carpos “fruit”. Several members of this genus are used in the preparation of herbal medicines, insect deterrents, and also fish poisons. *C. tomentosa* is a medium-sized tree reaching a height of up to 5 m, simple green, velvety tomentose, elliptic to ovate leaves of about 10-25 x 5-15 cm with long pointed apex. It is found mainly in the Western and Eastern Ghats region of India, Sri Lanka, Indonesia, Myanmar, Nepal, and the Malay Peninsula (Ray *et al*., 2022).

In traditional medicine, various parts of this plant are used to treat fever, liver injury, skin infections, and mouth ulcers. The juice mixed with common salt is administered as an anthelmintic, whereas the seeds are used to treat blood loss, hydrocele, malaria, and swellings. An aqueous leaf extract is used as an antiseptic to treat wounds, boils, asthma, cough, and ulcers. A decoction of the fruit is used to cure bleeding piles and the bark is being used in the East Indies as a substitute for betel leaf (Ray *et al*., 2022).

In Scientific Perspectives of Pandavara Batti, decoction of the bark used in fever, hepatic obstruction and skin diseases. Leaves boiled in milk and used as wash for aphthae of mouth. The fruits are used in epilepsy, diseases of nervous system, haemorrhage, oedema, cardiac disease and dysuria. Zulfkar *et al*.,(2014) stated that *Callicarpa linata* (Verbenaceae), an plant known worldwide as *Tomex tomentosa* L., it is widely used in tropical countries as a source of ethno medicines. It is recommended in a wide range of ailments including fever, hepatic obstruction, hepatic eruption, skin diseases.

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Nutritive value of *Callicarpa* (Beautyberry) is high in vitamins and minerals like vitamin C. It is high in carbohydrates and fiber. There are also rich antioxidants that give the berries that bright colour. Avoid eating too many raw berries, it can cause stomach problems. Antioxidants act as scavengers preventing cellular and membrane damage. Antioxidants act in different ways by preventing free radical formation, by scavenging free radicals, by preventing the propagation of the oxidative chain reaction, by being part of the redox antioxidant network, or by regulating gene expression. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. Leaves extract of *Callicarpa* showed significant antioxidant activity against various free radicals (Zulfkar *et al*., 2014).

Umachandur *et al.*, (2015) stated that *Calliacarpa* plants are a source of potential antibacterial and antifungals which may provide a solution to the problem. It has been a source of antimicrobials and extracts from about 14 species in this genus have been evaluated for biological activity, including antibacterial, antifungal activities.

According to the previous study, the genus showed a lot of important pharmacological uses in ancient and recent time. So, the objectives of this study is to find whether the leaf extracts of *Calliacarpa tomentosa* have nutritive value, antioxidant and antimicrobial activities or not.

Materials and Methods

1. Sample Collection, Preparation & Plant Identification

The leaves of *Callicarpa tomentosa* plant was collected from University of Myitkyina campus, during the period of 2021 to 2023. The samples were shade dried and powdered to fine texture and stored in the air tight containers. These powdered materials were used for experiments. The plant was identified with the available literatures of "List of Trees, Shrubs, Herbs and Principle Climbers" (Hundley *et al*., 1987) and "A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar" (Kress *et al*., 2003) and. Thet Thet Mar Win *et al*., 2019 (Useful Flowering Plants in Myanmar Vol.1). The collected sample leaves were washed with water and dried in air. The dried leaves were made into powder by using grinding machine. The powdered sample was stored in air-tight container to prevent moisture and contaminations. The dried powdered sample was used for investigating of activities.

2. Determination of Nutritive Value

The experimental work for the nutritional compositions of leaves powder was carried out at the Department of Physiology and Biochemistry, University of Veterinary Science, Yezin, Myanmar. The nutritional value has been undertaken according to the Association of Official Analytical Chemist (AOAC) (Horwitz, 1980).

3. Determination of Antioxidant Activity by DPPH

The antioxidant activity of dried leaves was evaluated by using DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay (Blosis, 1958 & Brand-Williams *et al*., 1995). This assay has been widely used to evaluate the free radical scavenging activity. The tests were conducted at Department of Chemistry, University of Mandalay. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colourless ethanol solution.

Table (1): Radical scavenging activity of various concentrations and IC_{50} value of standard ascorbic acid

Sample concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
200	0.079	85.1782	24.44
100	0.159	70.1689	
50	0.204	61.7261	
25	0.265	50.2814	
12.5	0.299	43.9024	
6.25	0.306	42.5891	

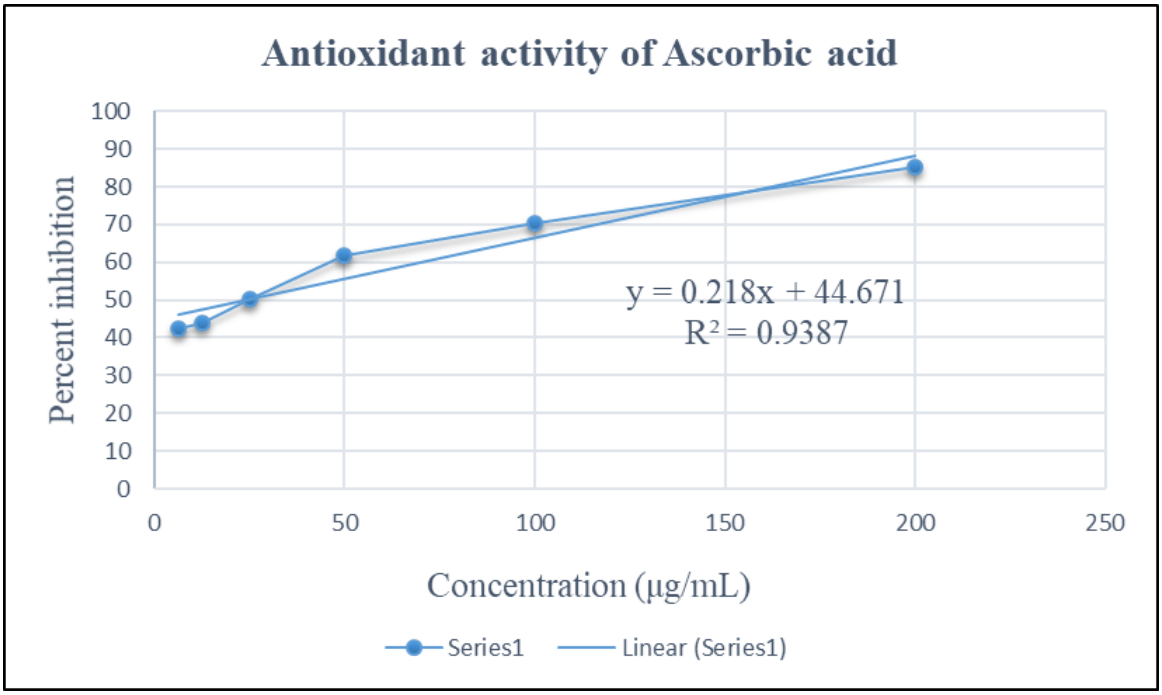

Figure 1. Plot of concentration Vs % inhibition of standard ascorbic acid

Chemicals

DPPH, Sample, 95% Ethanol, Ascorbic acid

Preparation of 0.002% DPPH solution

2 mg (0.002 g) of DPPH powder and 100 mL of 95% ethanol were thoroughly mixed by vortex mixer. This solution was freshly prepared in the brown coloured flask. Then it must be stored in the freezer for no longer than 24 hours.

Preparation of test sample solution

2 mg of test sample and 10 mL of 95% ethanol were thoroughly mixed by vortex mixer. The mixture solution (200 μgmL^{-1}) was diluted with 95% ethanol to obtain 100 μgmL^{-1}, 50 μgmL^{-1}, 25 μgmL^{-1}, 12.5 μgmL^{-1}, 6.25 μgmL^{-1}.

Preparation of ascorbic acid solution

2 mg of ascorbic acid was weighed and dissolved in 10 mL of 95% ethanol. The mixture solution (200 μgmL^{-1}) was diluted with 95% ethanol to obtain 100 μgmL^{-1}, 50 μgmL^{-1}, 25 μgmL^{-1}, 12.5 μgmL^{-1}, 6.25 μgmL^{-1} respectively (Table 1 & Figure 1).

Measurement of DPPH radical scavenging activity by spectrophotometric method (Blosis, 1958 & Brand-Williams *et al*., 1995). The control solution (A) was prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of 95% ethanol using vortex mixer. The test sample solutions (B) were prepared by mixing 1.5 mL of 0.002% DPPH solution and each 1.5 mL of sample solutions with various concentration. After that the solutions were allowed for 15 minutes in room temperature. Then the absorbance values were measured at 517 nm by UV Spectrophotometer. Similarly, each 1.5 mL of ascorbic acid solutions with different concentration was mixed with 1.5 mL of 0.002% DPPH solution and allowed for 15 minutes in room temperature. Then the absorbance values were measured at 517 nm by UV Spectrophotometer. The absorbance values obtained were applied to calculate percent inhibition by the following formula.

$$\% \text{ inhibition} = (A - B)/A \times 100\ \%$$

% Inhibition = percent inhibition of test sample

A = Absorbance of control solution

B = Absorbance of test sample solution (Absorbance of ascorbic acid solution)

4. Determination of Antimicrobial activity

Three different extracts were tested against seven pathogenic microorganisms; three gram positive bacteria; *Staphylococcus aureus* and *Bacillus pumilus* and *Bacillus subtilis* and three gram negative bacteria; *Escherichia coli*, *Pseudomonas fluorescens* and *Agrobacterium tumefaciens* and a fungal strain *Candida albicans* were used. By using agar-well diffusion method (Cruickshank *et al*. 1975) and conducted at the University's Research Centre, University of Myitkyina**.**

Results and Discussion

1. Plant Identification

Scientific name - *Callicarpa tomentosa* (L.) L.

Myanmar name - Daung-satpya

Vernacular name - Kyun nalin

English name - Velvety beauty berry

Family - Lamiaceae

It is a small tree with about 5m tall. Leaves are simple, opposite; densely white stellate tomentose, elliptic to broadly elliptic; apex acute or acuminate. Purplish flowers show branched axillary cymes. Fruit is 3-4 seeded black globose drupe. Fruits and leaves provide food for wildlife (Figure 2 A & B).

Figure 2. A. Habit as seen B. Flower

2. Nutritive Value

According to the experimental results, moisture, ash, crude protein, crude fiber, crude fat, carbohydrate was reported for *C. tomentosa.* The results were shown in Table (2).

Among them, crude fibre content was found to be the highest value (31.67%) and Non-structural carbohydrates were found to be (24.66%) and crude protein was found to be 19.63%. Sharma *et al*., 2021 investigated that physical constants of *C. macrophylla*, the total ash value was found to be 7.25 %. The present result of ash content was 9.49%. According to present data, it can be observed that the leaves contain many nutritional values which have benefits to humans.

3. Antioxidant Activity

According to the result of percentage of radical scavenging activity (% RSA), from leaves extracts are 59.66, 56.28, 45.40, 35.45, 34.52 and 33.77 at various concentration of 200, 100, 50, 25,12.5 and 6.25 µg/mL respectively. IC_{50} value of the *C. tomentosa* leaves extract is 105.96 µg/mL. IC_{50} value of the standard ascorbic acid is 24.44 µg/mL (Table 3 & Figure 3).

Zulfkar *et al*., (2014) stated that DPPH radical scavenging activity of ethanol extract of *C. linata* demonstrated in a concentration dependent manner and the IC_{50} was found to be 139 µg/mL. A positive DPPH test suggests that the extract is a potential free radical scavenger.

The antioxidant activity was confirmed by a decrease in absorbance band upon increasing concentrations of the ethanolic extract of *C. linata*. Sumon *et al*., (2019) found that IC_{50} value of the methanol extract of the *C. americana* leaves sample was 311.82 µg/mL while standard was 65.688 µg/mL. This result indicates the presence of antioxidant activity which is less significant.

Table (2): The results of nutritive value of leaves powder

Sr. No	Test parameter	Results (% in dry matter)
1	Moisture	12.86
2	Ash	9.49
3	Crude Protein	19.63
4	Crude Fibre	31.67
5	Fat	0.87
6	Non-structural Carbohydrate	24.66

Table (3): The results of % Inhibition and IC_{50} value

Sample concentration (µg/mL)	Absorbance	% inhibition	IC_{50} (µg/mL)
200	0.215	59.66229	
100	0.233	56.28518	105.96
50	0.291	45.40338	
25	0.344	35.45966	
12.5	0.349	34.52158	
6.25	0.353	33.77111	

According to Ray *et al*., (2022) the antioxidant potential of *C. tomentosa* essential oil was evaluated by DPPH assay. The sample was found to be higher in ascorbic acid (6.65 ± 0.30 µg/mL), followed by *C. tomentosa* leaf essential oil (17.12 ± 0.36 µg/mL). A lower IC_{50} value indicates a higher antioxidant activity. Sharma *et al*.(2021) observed that, in *C. macrophylla* leaves, percentage inhibition of standard ascorbic acid 23.44 µg/mL, ethyl acetate 72.62 µg/mL, and ethanol extract 55.64 µg/mL by DPPH assay. A study reported in 2016, by Sharma and his coworkers showed that methanol extract of the stem and leaves of *C. arborea* has an IC_{50} value of 53.65 and 47.20 µg/mL.

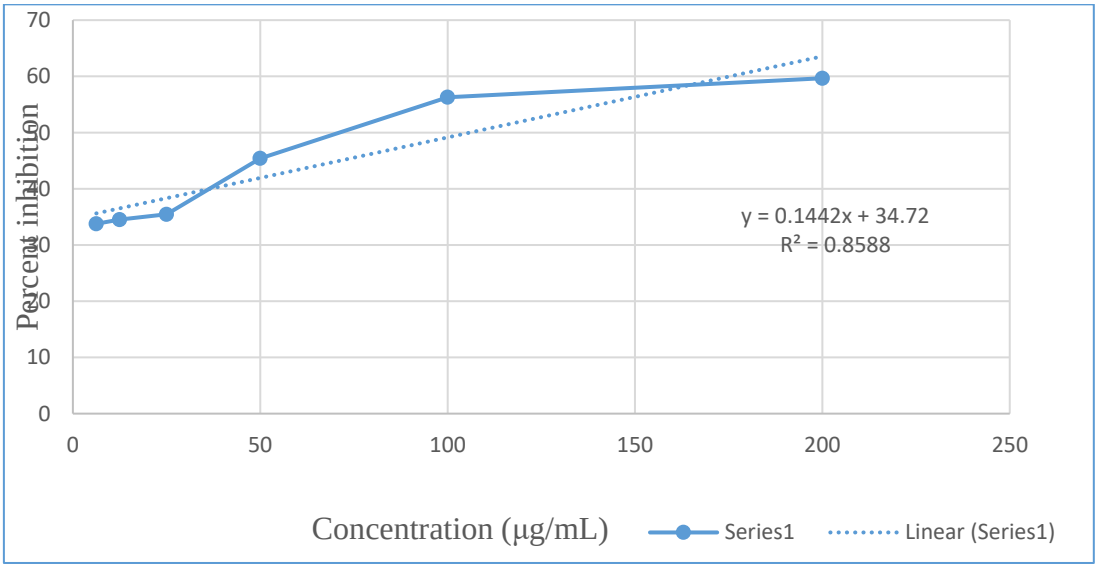

Figure (3): Plot of concentration Vs % inhibition of ethanol extract

3. Antimicrobial Activity

The results of antimicrobial activities were compared with control and were shown in Table 4 & Figure 4.

According to results, ethanol and aqueous extracts showed the most significant antimicrobial activity against *Agrobacterium tumefaciens* (40 mm) and ethanol extracts showed the significant antimicrobial activity against *Escherichia coli* (26 mm). Likely aqueous and methanol extracts showed the significant antimicrobial activity against *Agrobacterium tumefaciens* and *Escherichia coli* (23 mm) and ethanol, aqueous, methanol extracts showed maximum zone of inhibition against *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Bacillus pumilus* (20 mm). All extracts showed medium zone of inhibition against *Bacillus pumilus* & *Pseudomonas fluorescens* (15 mm and 13 mm). Only methanol extracts showed zone of inhibition against *Candida albicans* (18mm). Surprisingly all extracts did not show any inhibitory activity against *Bacillus subtilis*.

Umachandur *et al*.,(2015) mentioned that the hydro alcoholic extract of the leaves of *C. arborea* exhibited significant antifungal activity and, antibacterial activity against both gram positive and gram negative bacteria, whereas the extracts exhibited a zone of inhibition of 24-28 mm in gram negative and gram positive bacteria.

Sumon *et al*.,(2019) in *C. americana*, the antimicrobial potential was especially showed against *E. coli* and *B. subtilis*, methanol extract of plant 12.333±1.17 mm and 24±1 mm.Yadav *et al*., 2012, *C. macrophylla* stem extracts results shown that ethanolic extract (SEE) is active against both gram positive and gram negative bacterial strains. Maximum zone of inhibition of 17mm was obtained when SEE reacted with *Bacillus subtilis*. Surprisingly, aqueous extract (SAE) is inactive against all the bacterial strains except against gram negative *Salmonella typhimurium*. So, the different extracts of genus *Callicarpa* from different parts showed varying degrees of antimicrobial activity.

Table 4. The results of antimicrobial activities

No.	Microorganisms	Zone of Inhibition Zone (Diameter in mm)			Control		
		EtOH	Aqueous	MeOH	EtOH	Aqueous	MeOH
1.	*Agrobacterium tumefaciens*	40 (+++)	23 (+++)	40 (+++)	-	-	-
2.	*Pseudomonas fluorescens*	20 (+++)	15 (++)	13 (++)	-	-	-
3.	*Escherichia coli*	26 (+++)	-	23 (+++)	-	-	-
4.	*Candida albicans*	-	-	18 (+++)	-	-	-
5.	*Staphylococcus aureus*	-	-	20 (+++)	-	-	-
6.	*Bacillus subtilis*	-	-	-	-	-	-
7.	*Bacillus pumilus*	15 (++)	20 (+++)	-	-	-	-

(+) inhibition (-) no inhibition Agar well - 8mm

8mm – 12 mm (+) 13mm – 17 mm (++) 18mm above (+++)

Conclusion

The findings of the study show that *C. tomentosa* leaf extracts can be used as an antioxidant. It is clear that from the present study, leaf extracts have antimicrobial activity that effectively inhibit the growth of gram negative bacteria tested. Thus, it may be concluded that it contains natural compounds that could be a potential usage and this plant is significantly used for the treatment and prevention of diseases. Further studies can be conducted on secondary metabolites to explore more activities.

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