Indian Journal of Natural Products and Resources
Vol. 11(4), December 2020, pp. 244-249

Antioxidant and antitubercular activities of leaf extracts of *Canthium dicoccum* (Gaertn.) and *Amischophacelus axillaris* (L.)

K. S. Meghashree, K. P. Latha* and H. M. Vagdevi

Department of PG Studies and Research in Chemistry, Sahyadri Science College, Shivamogga 577203, Karnataka, India

Received 12 December 2019; Revised 16 October 2020

Modern civilization is facing hundreds of disorders associated with free radicals. The natural antioxidants from non-edible plants are gaining importance to fight against these disorders. One such commonly seen disorder is tuberculosis, which is responsible for about 8 million deaths annually worldwide. This study intends to evaluate the ethanol extracts of *Canthium dicoccum* (Gaertn.) and ethyl acetate extracts of *Amischophacelus axillaris* (L.) for antioxidant and antitubercular activities. The antioxidant activity of the extracts has been evaluated using DPPH radical scavenging methods. The results of the study indicated that ethanol extract of *C. dicoccum* (Gaertn.) and ethyl acetate extract of *A. axillaris* (L.) possess promising DPPH radical scavenging activity. The antitubercular activity of ethanol extract of *C. dicoccum* (Gaertn.) and ethyl acetate extract of *A. axillaris* (L.) have been evaluated against *Mycobacterium tuberculosis* H73Rv strain using Microplate Alamar Blue Assay (MABA). The activity was documented within the MIC range of 0.8 to 12.5 µg/mL for *C. dicoccum* (Gaertn.) and 0.8 to 50 µg/mL for *A. axillaris* (L.). The results of MABA showed that both the plant extracts exhibited excellent antitubercular activity. The present investigation suggests that *C. dicoccum* (Gaertn.) and *A. axillaris* (L.) possess remarkable antioxidant and antitubercular activity.

Keywords: *Amischophacelus axillaris* (L.), Antioxidant, Antitubercular, *Canthium dicoccum* (Gaertn.), Ethanol extract, Ethyl acetate extract.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/74, A61K 127/00, A61P 31/00, A61P 31/06, A61P 39/00

Introduction

Oxygen is essential for aerobic metabolism[1]. Oxidative stress increases due to the imbalance between antioxidant systems in the organism. A metal pollutant and high energy physical radiation is the main cause of the increase of free radical. The metal ions like copper, iron and cadmium which can donate their electron are to create ROS through a common Fenton reaction[2]. The increase of ROS will lead the way to many oxidative damages because of the oxidation of protein, lipid and nucleic acids. This occurrence results in various diseases such as age-related neurodegenerative diseases and cancer[3]. An antioxidant is a substance that can scavenge the free radical by inhibiting the beginning step, thus bring down oxidative stress. Even though some synthetic antioxidants were used in plenty of food industries, antioxidant like butylated-hydroxytoluene (BHT) was demonstrated to be carcinogenic[4]. Alternative antioxidants derived from natural sources must be explored. Tuberculosis (TB) is one of the foremost infectious disease and a global health burden[5]. It has been evaluated that, one-third of the world's population is currently infected with tuberculosis[6]. TB is one of the top three infectious killing diseases in the world. HIV/AIDS kills 3 million people each year, TB kills 2 million and malaria kills 1 million[7]. The involvement of TB with HIV infection is so prevalent that nearly two-thirds of the patients diagnosed with tuberculosis are also HIV-1 seropositive[8]. Antitubercular drugs such as rifampicin (RIF), ethambutol, isoniazid (INH), streptomycin, etc have been a foundation in the healing of tuberculosis[9]. The screening of plant extracts has been of great importance to scientists in the search for novel drugs for the helpful treatment of several diseases[10]. Based on the two problems foregoing, plants have been considered to possess a diverse agent of antioxidant and antitubercular substances. Currently, the use of alternative medicine starts to replace conventional medicine. *Canthium dicoccum* (Fig. 1), found in the Western Ghats of India is commonly known as Ceylon boxwood in English and as nallabalusu in Telugu, belongs to the family *Rubiaceae*[11]. In India, its bark is used to cure fever and a decoction of the root is used internally for diarrhea. Bark powder with sesame oil is used in rheumatic pain[12,13]. The plant is proved for its

*Correspondent author
Email: lathakp337@gmail.com

Fig. 1 — *Canthium dicoccum* (Gaertn.).

anti-inflammatory activity[11], antidiabetic and nephroprotective activity[14]. The bark contains sitosterol, quinovaic acid, acetylquinovaic acid and scopoletin[15]. Leaves contain ursolic acid, rutin, quercetin, 7-O-(6-O-benzoyl- β-glucopyranosyl)-rutin, caryophyllene oxide (19.25%), spathulenol (20.76%), cedren-13-ol (10.62%) and ledene oxide (5.24%)[12]. *Amischophacelus axillaris* (L.) (Fig. 2) belongs to the family *Commelinaceae* commonly called Negiluthare in Kannada. It is native to the Indian Subcontinent, southern China, Southeast Asia, and Northern Australia. Traditionally plays an important role in anti-inflammatory, febrifuge, antiparasitic and antifungal activities. In India, leaves are used for the treatment of tympanitis and as food for pigs[16].

Current studies have indicated the need for the development of new, safe, and efficacious drugs to help reduce the global burden of tuberculosis, prevent oxidative damage. Natural products of plant biodiversity have received considerable attention as potential agents since they are a proven template for the development of new molecules against tuberculosis. Many antitubercular compounds that may prove to be useful leads for drug discovery have been derived from medicinal plants[17]. Natural products, especially those from the plant biodiversity have been less intensively investigated in the past even though they are known to contain structurally diverse molecules, many of which are unknown. This has prompted the researchers to undertake the current investigation of medicinal plants for their anti-TB, antioxidant activities.

Material and Methods

Plant material

The *C. dicoccum* (Gaertn.) leaves were collected in the month of June-July (2018) in Hosnagar (T), Shimoga district, Karnataka, and *Amischophacelus axillaris* (L.) leaves were collected in the month of July-August (2018) in Agumbe region, Shimoga district, Karnataka. Both the plants were authenticated by Dr. Geetha, Ayurvedic Doctor (BAMS), Thrinive, Hosanagar (T), Shivamogga (D) and deposited in the Department of Botany Kuvempu University, Shanakaragatta, with the voucher number KUAB4688 for *C. dicoccum* (Gaertn.) and KUAB4687 for *A. axillaris* (L.). The collected plant material was shade dried and coarsely pulverized.

Fig. 2 — *Amischophacelus axillaris* (L.).

Preparation of extract

The pulverized plant material was subjected to the using Soxhlet extractor. The extraction was carried out using numerous solvents viz., pet ether, ethyl acetate, and ethanol as per their increasing polarity.

The obtained extract was filtered and evaporated to dryness under reduced pressure in a rotary vacuum evaporator. Among all the successive extracts, ethanol extract of *C. dicoccum* (Gaertn.) and ethyl acetate extract of *A. axillaris* (L.). leaves were used for *in vitro* activities.

***In vitro* antioxidant assay**

The antioxidant activity of plant extracts was determined by *in vitro* method, the DPPH (2,2-Diphenyl-1-picryl hydrazyl) free radical scavenging activity and total phenolic content assay. DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al*.,[18]. The different concentrations of each of the extracts were prepared in methanol and were added to 3 mL of 0.1 mm methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 minutes at room temperature in dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\text{Percentage of inhibition} = \frac{\text{OD of control - OD of test}}{\text{OD of control}} \times 100$$

where OD is the Optical Density.

Antitubercular activity

The antimycobacterial activity of the extracts was assessed against *M. tuberculosis* H37Rv Strain using microplate Alamar Blue assay (ATCC No- 27294). This methodology is non-toxic, uses a thermally stable reagent, and shows a good correlation with BACTEC radiometric methods. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds in ethanol extract of *C. dicoccum* (Gaertn.) and ethyl acetate extract of *A. axillaris* (L.) was made directly on the plate. The final drug concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25 µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hours[19]. *Streptomycin* was used as a standard drug**.**

Results

DPPH free radical scavenging activity

DPPH radical is one of the constant and commercially existing organic nitrogen radicals[20,21,22]. This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is comparative to the disappearance of DPPH radicals in test samples. DPPH radical shows a well-built absorption maximum at 517 nm (purple). A newly prepared DPPH solution shows a deep purple colour with an absorption maximum at 517 nm. The purple colour generally fades when an antioxidant is present in medium[23,24]. Table 1 shows the ethanol extract of *C. dicoccum* with potent antioxidant activity at the concentration 1000 µg/mL showing a value of 85.42±0.096 µg/mL and the Table 2 shows the ethyl acetate extract of *A. axillaris* (L.) with potent antioxidant activity at concentration 1000 µg/mL

Table 1 — Antioxidant activity of leaves extracts of *Canthium dicoccum* (Gaertn.)

Extracts	Concentration µg /mL	DPPH (%) inhibition
Ethanol	250	54.21±1.477
	500	57.28±0.096
	750	72.19±0.096
	1000	85.42±0.096
Ascorbic acid	250	63.80±0.059
	500	69.35±0.059
	750	77.34±0.059
	1000	81.87±0.059

Significance level: The data were analyzed using one way ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at P <0.05*, P <0.01**, *C. dicoccum*: *Canthium dicoccum*, SEM: Standard error of the mean, ANOVA: Analysis of variance, DPPH: 2, 2-diphenyl-picryl-hydrazyl

Table 2 — Antioxidant activity of leaves extracts of *Amischophacelus axillaris* (L.)

Extracts	Concentration µg /mL	DPPH (%) inhibition
Ethylacetate	250	51.59±0.147
	500	61.13±0.096
	750	69.84±0.096
	1000	82.74±0.096
Ascorbic acid	250	63.80±0.059
	500	69.35±0.059
	750	77.34±0.596
	1000	81.87±0.059

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at P <0.05*, P <0.01**, *A. axillaris* (L.): (L.), SEM: Standard error of the mean, ANOVA: Analysis of variance, DPPH: 2, 2-diphenyl-picryl-hydrazyl.

Table 3 — Antituberculer activity of *Canthium dicoccum* (Gaertn.) and *Amischophacelus axillaris* (L.)

S. No	Sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL
1	*C.dicoccum*	S	S	S	S	R	R	R
2	*A.axillaris*	S	S	R	R	R	R	R
3	*Streptomycin*	S	S	S	S	S	R	R

S- Sensitive, R-Resistance, Standard- *Streptomycin*

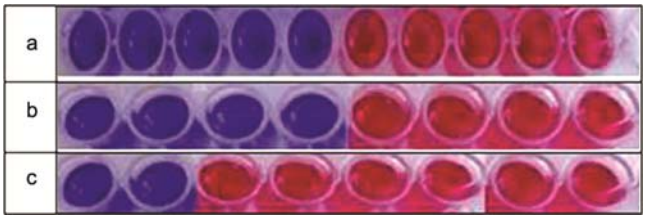

Fig. 3 — Antitubercular activity a) Standard, b) Ethanol extract of *Canthium dicoccum*, and c) Ethyl acetate extract of *Amischophacelus axillaris.*

showing a value of 82.74±0.096 µg/mL, respectively. These activities are comparable with the standard ascorbic acid, which has a scavenging effect with the percentage of inhibition 81.87±0.059.

Antitubercular activity

For anti-TB activity using Alamar Blue Dye, blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as the lowest drug concentration which prevented the colour change from blue to pink. The ethanol extract of *C. dicoccum* (Gaertn.) showed a MIC of 12.5 µg/mL and ethyl acetate extract of *A. axillaris* (L.) showed a MIC of 50 µg/mL by preventing the colour change from blue to pink tabulated in Table 3 and Fig 3. The anti-TB activity of the extracts was comparable to that of the standard drug *streptomycin* concentration of 6.25 µg/mL by interpreting data of MIC. The preliminary evaluation of the crude extracts of *C. dicoccum* and *A. axillaris* showed that both the plants are a potential source of bioactive compounds that can inhibit the antioxidant and anti-TB activities**.** Further purification studies will be carried out to identify the bioactive compounds responsible for the observed activity.

Discussion

Antioxidant activity

Several *in vitro* assays are used to assess the radical scavenging potential of samples. Among them, DPPH free radical scavenging assay is one of the most extensively used assays. DPPH is a firm, nitrogen centred, organic free radical having an absorption maximum at 517 nm in alcoholic solution. The radical becomes a stable diamagnetic molecule on accepting a hydrogen atom from the antioxidant. In the occurrence of a donor capable of donating hydrogen atom, the radical nature of DPPH is lost and its colour (purple) changes to yellow (diphenyl-picrylhydrazine). The solution loses colour stoichiometrically depending on the number of electrons taken up. This assay has been used to study radical scavenging activity of various kinds of samples including plant extracts[25-29]. In the present study, noticeable scavenging efficacy was observed in the case of the extract of *C. dicoccum* (Gaertn.) and *A. axillaris* (L.). The results obtained has P values less than 0.05 it means that the result is statistically significant. The scavenging effect of extracts was higher than that of ascorbic acid. It is clear from the study that the extracts possess hydrogen donating ability and hence, these extracts can act as free radical scavengers, acting possibly as primary antioxidants[26].

Antitubercular activity

Literature reports revealed a large set of natural products having anti TB properties, 80% of the natural products under study have the unique capability to counter the deadly tuberculosis[30]. Current TB therapy consists of treatment with a combination of drugs. This combination of therapy causes hepatotoxicity as the major side effect as well as the development of drug resistance. To avert toxicity and reduce the ineffectiveness of current anti-TB drugs, medicinal plants are considered as potential anti-tuberculosis agents that can be used in combination with the standard anti-tuberculosis drugs or alone[31]. In this study, the preliminary evaluation of the extracts of *C. dicoccum* and *A. axillaris* showed that both the plants are a potential source of bioactive compounds that can inhibit the anti-TB activity. Accordingly, the ethanol fraction was found to be the most active fraction, based on MIC values (12.5 µg/mL), which indicated that antimycobacterial constituents were contained in this fraction.

Conclusion

This study investigated the potential of *C. dicoccum* and *A. axillaris* as a new plant source

exhibiting antioxidant and antitubercular activities. The outcome of these activities revealed that the ethylacetate and ethanol extracts exhibited a significant amount of activity, which is generally due to the active phytoconstituents present in the extracts. The present study gives evidence that it may be a very effective medicine in upcoming days. Further, the plant extracts will be explored for its phytochemical outline to identify the active component, which is responsible for antioxidant and anti tubercular activities.

Conflict of interest

There is no conflict of interest.

References

1 Mittler R, ROS are good, *Trends Plant Sci*, 2017, **22**(1), 11-19.

2 Valko M, Jomova K, Rhodes C J, Kuca K and Musilek K, Redox-and non-redox-metal-induced formation of free radicals and their role in human disease, *Arch Toxicol*, 2016, **90**(1), 1-37.

3 Thanan R, Oikawa S, Hiraku Y, Ohnishi S, Ma N, *et al*., Oxidative stress and its significant roles in neurodegenerative diseases and cancer, *Int J Mol Sci*, 2014, **16**(1), 193-217.

4 Mark J, Pollien P, Lindinger C, Blank I and Mark T, Quantitation of furan and methylfuran formed in different precursor systems by proton transfer reaction mass spectrometry, *J Agric Food Chem*, 2006, **54**(7), 2786-2793.

5 Dye C, Scheele S, Dolin P, Pathania V and Raviglione M C, Global burden of tuberculosis: Estimated incidence prevalence and mortality by country, *JAMA*, 1999, **282**, 677-686.

6 Gupta R, Thakur B, Singh P, Singh H B, Sharma V D, *et al.*, Anti-tuberculosis activity of selected medicinal plants against *Mycobacterium tuberculosis*, *Ind J Med Res*, 2010, **131**, 809-813.

7 Gizachew Y E, Giday M and Teklehaymanot T, Antimycobacterial activity of selected *Ethiopian* traditional medicinal plants used for the treatment of symptoms of tuberculosis, *Glo Adv Res J Med Plants*, 2013, **2**, 22-29.

8 Ilango K and Arunkumar S, Synthesis, antimicrobial and antitubercular activities of some novel trihydroxybenzamido azetidine-2one derivatives, *Trop J Pharm Res*, 2011, **10**, 219-229.

9 Panda V S, Ashar H D and Sharan A, Antioxidant and hepatoprotective effects of *Garcinaindica* fruit rind in antitubercular drug-induced liver injury in rats, *Botanics: Targets and therapy*, 2013, **3**, 29-37.

10 Dimayuga R E and Garcia S K, Antimicrobial screening of medicinal plants from *Baja California sur*, Mexico, *J Ethnopharmacol*, 1991, **31**(2), 181-192.

11 Bhaargavi V, Reshma T, Jyotsna G and Firasat Ali, Anti-inflammatory activity of ethanolic extract of *Canthiumdicoccum*, *Int J Pharm Phytopharm Res*, 2013, **3**, 226-230.

12 Raja Rajeswari N, Ramalakshmi S and Muthuchelian K, GC-MS analysis of bioactive components from the ethanolic leaf extract of *Canthiumdicoccum* (Gaertn.) Teijsm & Binn, *J Chem Pharm Res*, 2011, **3**, 792-798.

13 Neelima M, Prasad G P, Sudarsanam G, Penchala P G, Jothi B, *et al*., Ethnobotanical studies in Rapur forest division of Nellore district in Andhra Pradesh, *Life Sci Leafl*, 2011, **11**, 333-345.

14 Santhan S, Janarthan M and Zuber ali M, Evaluation of antidiabetic and nephroprotective activity of 95% ethanol extract of *Canthium dicoccum* whole plant by using albino rats, *J Chem Pharm Sci*, 2013, **6**, 218-222.

15 Herath W H, Sultanbawa M U S, Wannigama G P and Andre C, Alkaloidal and other constituents of Uncariaelliptica and *Canthium dicoccum*, *Phytochem*, 1979, **18**,1385-1387.

16 Thorn G W, Adams R D, Brauwald E K J, Isselbacher, Petersdoft R G, *et al*., *Harrison's Principles of Internal Medicine*, (McGraw Hill and Company), 1977, **10**, 1088.

17 Nguta J M, Appiah-Opong R, Alexander K N, Yeboah-Manu D, Phyllis G A, *et al*., Antimycobacterial and cytotoxic activity of selected medicinal plant extracts, *J Ethnopharmacol*, 2016, **182**, 10- 15.

18 Wong S P, Lai P L and Jen H W, Antioxidant activities of aqueous extracts of the selected plant, *Food Chem*, 2006, **99**, 775-83

19 Lourenco M C S, de Souza M V N, Pinheiro A C, Ferreira M D L, Gonçalves R S B, *et al.*, Evaluation of the anti-tubercular activity of nicotinic and isoniazid analogs, *ARKIVOC*, 2007, **15**, 181-191.

20 Cuendet M, Hostettmann K and Potterat O, Iridoid glucosides with free radical scavenging properties from *Fagraeablumei*, *Helv Chim Acta*, 1997, **80**, 1144–1152.

21 Burits M and Bucar F, Antioxidant activity of *Nigella sativa* essential oil, *Phytother Res*, 2000, **14**, 323-328.

22 MacDonald-Wicks L K, Wood L G and Garg M L, Methodology for the determination of biological antioxidant capacity *in vitro* a review, *J Sci Food Agric*, 2006, **86**, 2046-2056.

23 Brand-Williams W, Cuvelier M E and Berset C, Use of a free radical method to evaluate antioxidant activity, *Lebensm Wiss Technol*, 1995, **28**, 25-30.

24 Mensor L L, Menezes F S, Leitao G G, Reis A S, Dos Santos T C, *et al*, Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, *Phytother Res*, 2001, **15**, 127-30.

25 Devasagayam T P A, Boloor K K and Mishra K P, Some new methods for free radical research, *SFRR-India Bulletin*, 2003, **2**, 20-28.

26 Chung Y, Chien C, Teng K and Chou S., Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides Sieb* and zucc, *Food Chem*, 2006, **97**, 418-425.

27 Kekuda P T R, Vinayaka K S, Swathi D, Suchitha Y and Venugopal T M, *et al*., Mineral composition, total phenol content and antioxidant activity of a macrolichen *Everniastrum cirrhatum (Fr.) Hale (Parmeliaceae*), *E J Chem*, 2011, **8**, 1886-1894.

28 Kekuda P T R, Manasa M, Poornima G, Abhipsa V, Rekha C, *et al.*, Antibacterial, cytotoxic and antioxidant potential of *Vitex negundo var. negundo* and *Vitex negundo var. purpurascens*- A comparative study, *Sci Technol Arts Res J*, 2013, **2**, 59-68.

29 Seruga M, Novak I and Jakobek L, Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and

spectrophotometric methods, *Food Chem*, 2011, **124**, 1208- 1216.

30 Kalpanaa A and Jayashreeb M, In silico analysis of the efficacy of some natural compounds as antituberculosis agents, *Indian J Chem*, 2020, **59**, 207-213.

31 Nair S S, Pharande R R, Bannalikar A S and Mukne A P, *In vitro* anti-mycobacterial activity of acetone extract of *glycyrrhiza glabra*, *J Pharm Pharmacogn Res*, 2015, **3**, 81.

32 Stagos D, Antioxidant activity of polyphenolic plant extracts, *Antioxidants*, 2020, **19**, 1-7.