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Research Paper

Evaluation of some pharmacological activities of Budmunchiamine - A isolated from *Albizia amara*

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Abstract

The present investigations were aimed to evaluate the antimicrobial and antioxidant efficacies of budmunchiamine-A (BUA) of *Albizia amara*. The activity-guided isolation leaded to isolate the bioactive compound budmunchiamine-A from alkaloid extract of *A. amara*. The budmunchiamine-A showed significant broad-spectrum antimicrobial activity with zone of inhibition (ZOI), minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) values varied from 7.3 to 24.5 mm, 0.95 to 62.5 μg/mL, and 1.9 to 250 μg/mL, respectively. The budmunchiamine-A exhibited moderate antioxidant activity with inhibitory concentration 50% (IC_{50}) value of 400 μg/mL in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and percent inhibition of β-carotene/linoleic acid was 67.8%. The results suggest the possible use of budmunchiamine-A as a molecular entity for drug development in pharmaceutical industry.

Key words: *Albizia amara*, budmunchiamine-A, antimicrobial activity, antioxidant activity.

Introduction

Infectious diseases are the major cause of death, accounting approximately one-half of all deaths in developing countries. The increasing reports on multidrug resistant pathogenic microbes to currently available drugs have further complicated the treatment of infectious diseases (Ahmad and Beg, 2001, Iwu *et al.*, 1999). Free radicals and reactive oxygen species (ROS) are produced in living cells as unwanted by-products during metabolic process, which cause oxidative stress related diseases like cancer and diabetes in human beings and oxidative reactions in food and pharmaceutical products (Ebrahimabadi *et al.*, 2010, Medina *et al.*, 2001). A number of synthetic antioxidants and free radical scavengers have been used to prevent ROS and oxidative reactions in food and pharmaceutical products (Kumaran and Karunakaran, 2007). Recent reports stated that the application of synthetic antioxidants in food processing has led to the appearance of remarkable side effects (Ebrahimabadi *et al.*, 2010). The increasing side-effects of synthetic antioxidants, and emergence of multidrug resistant pathogenic microbes are collectively necessitated to look for alternative strategies (Ahmad and Beg, 2001, Iwu *et al.*, 1999).

In recent decades, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being (Parekh and Chanda, 2007). Their role is crucial in the development of new drugs, they may become the base for the development of a medicine, a natural blueprint for the development of new therapeutic molecules (Iwu *et al.*, 1999, Kumaran and Karunakaran, 2007). The bioactive compounds of plant origin have been screened for their potential uses as alternative agents for the treatment of infectious diseases, preservation of food from microbial deterioration and the effect of oxidants (Dung *et al.*, 2008, Medina *et al.*, 2001, Reza *et al.*, 2010). Alkaloids are a structurally diverse group of over 12,000 cyclic nitrogen-containing compounds that are found in over 20% of plant species, which are important bioactive substances and have been reported for their various bioactivities (Ahmad *et al.*, 2006, Deng *et al.*, 2011, Medina *et al.*, 2001, Roy and Chatterjee, 2010).

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The *Albizia amara* (Leguminosae) is a small to moderate sized deciduous tree, is rich in alkaloids and grows in tropical regions of southern India. The leaves are used as folk remedy for curing various diseases *viz.,* stomach cancer, common cold, diarrhoea, intestinal ailments, dandruff, wounds, skin diseases, gonorrhoea and animal fodder (Ayyanar and Ignacimuthu, 2005, Kareru *et al.*, 2008). Some biological activities of aqueous and solvent extracts of *A. amara* are reported (Kareru *et al.*, 2008, Karmegam *et al.*, 2012, Khan *et al.*, 2010, Kumar *et al.*, 2010, Muchuweti *et al.*, 2006, Praveen *et al.*, 2011). Previous reports stated that the budmunchiamines are the main alkaloid constituents in this plant (Pezzuto *et al.*, 1991, Pezzuto *et al.*, 1992, Rajkumar and Sinha, 2010). The budmunchiamines mixtures have been reported for some biological activities (Mar *et al.*, 1991). Although, there are no reports on the antimicrobial activity against both Gram-positive and Gram-negative bacteria, and yeasts, and antioxidant activities of budmunchiamine-A (BUA). Hence, the present study has made an attempt to analyse the antimicrobial and antioxidant activities of BUA.

Material and Methods

Chemicals and culture media

Mueller-Hinton agar/broth (MHA/MHB), malt extract-glucose-yeast extract-peptone-agar/broth (MGYPA/MGYPB), dimethyl sulfoxide (DMSO), β-carotene, linoleic acid, neomycin (NM) and fluconazole (FZ) were purchased from Hi-Media, Mumbai (India). All solvents, reagents, ascorbic acid and iodo-nitro-tetrazolium (INT) were purchased from SRL, Mumbai. Microtiter-plates (96-well) were purchased from Axiva, New Delhi (India). 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma, Germany. Silica gel 60 F_{254}-coated preparative thin-layer chromatography (TLC) plates were obtained from Merck, Germany.

Plant sample collection

Fresh leaves of *Albizia amara* (Roxb.) B.Boivin were collected from the southern part of Karnataka (India) during 2010-12. The plant samples were authenticated by Dr. Sankara Rao, Professor, JCB National Herbarium and authenticated voucher specimens were deposited in JCB National Herbarium, Indian Institute of Science, Bangalore (India) (Voucher number: BUB/MB-BT/DCM/JU10/23).

Isolation and identification of bioactive compounds from alkaloid extracts

The leaves were shade-dried, powdered and used for alkaloid extraction following the procedure of Harborne (1998). The isolation of bioactive compound was done from the alkaloid extracts of *A. amara* as reported in our previous paper (Thippeswamy *et al.*, 2013). Briefly, the activity guided isolation of bioactive molecule from alkaloid extracts of *A. amara* was done initially by silica gel column chromatography and further purified by preparative TLC, and subjected to spectral analysis for structural elucidation. In IR spectrum, the bioactive compound showed characteristic absorption peaks at 1649.61, 3359.77, and 2945.54 confirmed the presence of strong C=O stretch, N-H stretch and alkane C-H stretch, respectively. In the positive mode $[M+H]^+$ electro-spray ionization mass spectroscopy, active compound showed molecular ion peak (*m/z*) at 453.88 corresponding to the molecular formula $C_{27}H_{56}N_4O$ (MW. 452.76), respectively. Further, based on NMR spectroscopic analysis and cited literature data (Pezzuto *et al.*, 1991, Pezzuto *et al.*, 1992), the isolated compound was identified as budmunchiamine-A (Figure 1).

Antimicrobial activities

Microbial strains

The pathogenic *Escherichia coli* (NCIM 2065), *Klebsiella pneumoniae* (NCIM 2957), *Proteus vulgaris* (NCIM 2027), *Pseudomonas aeruginosa* (NCIM 5031), *Salmonella typhi* (NCIM 2051), *Staphylococcus aureus* (NCIM 2079), *Streptococcus faecalis* (NCIM 5025), *Candida albicans* (NCIM 3471) and *Cryptococcus neoformans* (NCIM 3541) were obtained from the National Chemical Laboratory, Pune (India). All the tested bacteria and fungi were maintained on MHA and MGYPA, respectively. Twenty four hours old bacterial and 48 h old fungal cultures were used as test organisms.

Disc diffusion method

The disc diffusion method was employed for the determination of zones of inhibition (ZOI) according to the method described by Ebrahimabadi *et al.* (2010) with slight modifications. Briefly, sterile filter paper discs (6 mm in diameter) were individually impregnated with 20 µL of two-fold diluted BUA dissolved in DMSO (0.95 to 1000 µg/disc), placed onto the pre-inoculated

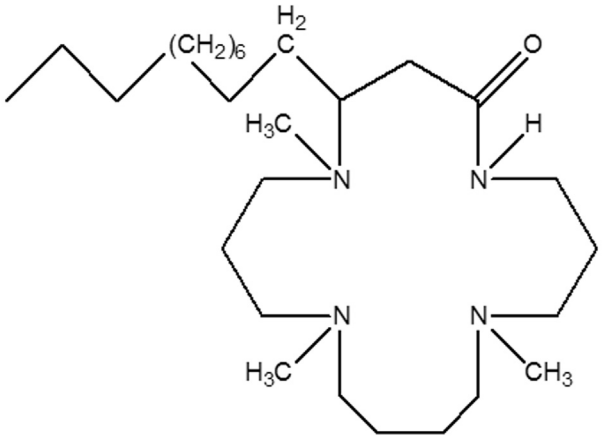

Figure 1 - Structure of budmunchiamine-A.

MHA/MGYP plates (inoculum size: 100 µL of a microbial suspension containing 10^8 cfu/mL of bacteria or 10^6 cfu/mL of fungi) and incubated at 37 °C for bacteria (24 h) and 30 °C for fungi (48 h). DMSO served as a negative control, and twofold diluted neomycin (for bacteria) and fluconazole (for fungi) served as positive controls. The ZOI diameters were measured in millimetres (mm).

Determination of the MICs and MBCs/MFCs

The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)/minimum fungicidal concentrations (MFCs) following the standard procedures with some modifications (Dung *et al.*, 2008, Hajji *et al.*, 2010). Briefly, 200 µL of two-fold serially diluted BUA in MHB or MGYPB (0.95 to 1000 µg/mL) was added to the wells of a sterile 96-well microtiter plate and inoculated with 15 µL of a microbial suspension containing 10^8 cfu/mL of bacteria or 10^6 cfu/mL of fungi, respectively, and incubated at 37 °C for bacteria (24 h) and 30 °C for fungi (48 h). DMSO served as a negative control, and neomycin and fluconazole were used as positive controls. After incubation, the MIC values of the compounds were detected by the addition of 50 µL of INT (2 mg/mL) according to the procedure of Hajji *et al.* (2010). MIC was defined as the lowest concentration at which no visible microbial growth was observed. The MBC/MFC values were determined following the procedure of Dung *et al.* (2008). Fifty microlitre of cultured broth (without INT) was radially streaked onto the MHA/MGYPA media and further incubated for respective time and temperature as mentioned above. The complete absence of growth on the agar surface at the lowest concentration was defined as the MBC/MFC.

Antioxidant activity

The antioxidant activity was determined by DPPH radical scavenging and β-carotene/linoleic acid assays (Ebrahimabadi *et al.*, 2010). In DPPH assay, two-fold dilutions of BUA was made using methanol (ranging from 31 to 1000 µg/mL). One millilitre of each dilution was mixed with 3 mL of freshly prepared methanol solution of DPPH (40 µg/mL) and incubated for 30 min in the dark at room temperature. The same concentration of ascorbic acid was used as positive control and methanol solution of DPPH served as negative control. The absorbance of the solutions was recorded using UV-Vis spectrophotometer (ELICO, SL-210 double beam spectrophotometer) at 517 nm. Percent inhibition of DPPH radical was calculated by following formula.

$$I\ \% = \{(A_{control} - A_{sample})/A_{control}\} \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test samples.

In β-carotene/linoleic acid assay, 500 µL of BUA (2000 µg in 1000 µL of methanol) was added to 2500 µL of β-carotene-linoleic acid emulsion mixture separately, mixed thoroughly and incubated at 50 °C for 2 h in water bath. Methanol was used as negative control and ascorbic acid was used as positive control. The absorbance was measured at 470 nm using UV-Vis spectrophotometer. The antioxidant activity (Inhibitions percentage) was calculated using following formula

$$I\% = (A_{\beta\text{-carotene after 2 h}}/A_{\text{initial }\beta\text{-carotene}}) \times 100$$

where, $A_{\beta\text{-carotene after 2 h}}$ is the absorbance of β-carotene after 2 h and $A_{\text{initial }\beta\text{-carotene}}$ is the absorbance of β-carotene at the beginning.

Statistical analysis

Data given of all tests were expressed as means of four replicates ± standard error. Analysis of variance was conducted, and the differences between values were tested for significance by ANOVA by applying Tukey's multiple comparison tests with the SPSS 20 (IBM, USA) programme. Differences at $p \leq 0.05$ were considered statistically significant.

Results and Discussion

As a result of the emergence of multidrug resistant bacteria and fungi, and the increasing occurrence of opportunistic systemic mycosis led renewed interest to search new bioactive molecules. In this way, we demonstrated the bioactive properties of active constituent BUA of *A. amara*, which is responsible for antimicrobial and antioxidant activities. The antimicrobial efficacy of bioactive compound was determined by employing disc diffusion and broth microdilution techniques. The isolated BUA showed a concentration-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria, and antifungal activity against *C. albicans* and *C. neoformans* (Table 1). The negative control DMSO did not inhibit any tested microorganisms. The BUA displayed significant activity against Gram-positive and Gram-negative bacteria with ZOI, MIC and MBC ranged 7.3-24.5 mm, 0.95-62.5 µg/mL and 1.9-250 µg/mL, respectively. Similarly, the antifungal activity with ZOI, MIC and MFC ranged 13.5-13.8 mm, 7.8 µg/mL and 15.6 µg/mL, respectively. Among the bacteria tested, the Gram-positive *S. faecalis* was the most susceptible species followed by *S. aureus*, whereas the Gram-negative *P. vulgaris* was the most resistant. The antibacterial and antifungal activities of the synthetic antibacterial (neomycin) and antifungal (fluconazole) agents were also determined and compared with the MIC and MBC/MFC values of BUA. The increasing order of the inhibitory activity against bacteria was BUA < neomycin and fungi was fluconazole < BUA.

Table 1 - Antimicrobial activity of BUA against bacteria and yeast.

Organisms	BUA			Neomycin/Fluconazole		
	ZOI[a]	MIC[b]	MBC[c]	ZOI[a]	MIC[b]	MBC[c]
E. coli	11.3 ± 0.3	3.9	15.6	16.8 ± 0.2	3.9	7.8
K. pneumoniae	10.1 ± 0.2	7.8	15.6	17.1 ± 0.2	1.9	3.9
P. vulgaris	7.3 ± 0.2	62.5	250	20.3 ± 0.3	1.9	3.9
P. aeruginosa	9.1 ± 0.2	15.6	31.2	16.6 ± 0.2	1.9	7.8
S. typhi	11.5 ± 0.3	7.8	31.2	19.5 ± 0.3	1.9	3.9
S. aureus	14.3 ± 0.2	15.6	31.2	19.5 ± 0.4	0.95	3.9
S. faecalis	24.5 ± 0.3	0.95	1.9	27.5 ± 0.3	0.95	1.9
C. albicans	13.8 ± 0.2	7.8	15.6	33.3 ± 0.4*	31.2	> 1000
C. neoformans	13.5 ± 0.3	7.8	15.6	35.7 ± 0.3*	15.6	500

Notes: [a]ZOIs at 0.25 mg/disc (mm); [b]MICs (μg/mL) & [c]MBCs/MFCs (μg/mL); Neomycin was used as positive control for bacteria and fluconazole for fungi; *fungistatic zone.

The DPPH radicals scavenging and β-carotene bleaching assays were used to determine the antioxidant potency of BUA. Antioxidants on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character (Dung *et al.*, 2008). The reduction in intensity of DPPH solution in the presence of antioxidants at 517 nm is usually taken as a measure of their antioxidant activity. Similarly, β-carotene/linoleic acid method is based on the loss of orange colour of β-carotene due to its reaction with radicals formed in linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be delayed down in the presence of antioxidant (Oke *et al.*, 2009). In the DPPH assay, the IC_{50} value was found to be 400 μg/mL, whereas the β-carotene/linoleic acid assay showed 67.8% inhibition of β-carotene/linoleic bleaching at 1000 μg/mL, and the activities were compared with the synthetic antioxidant ascorbic acid (Table 2). The increasing order of antioxidant activity was BUA < ascorbic acid.

The results of the present investigation confirmed the strong antimicrobial activity of BUA against Gram-positive bacteria than Gram-negative bacteria and yeast. These results are of great importance, particularly for *S. aureus,* which is well known for being resistant to a number of antibiotics (Dung *et al.*, 2008). Mar *et al.* (1991) have reported the bactericidal activity of mixture of budmunchiamines isolated from *A. amara*, against only one Gram-negative *S. typhimurium.* Although, this is the first report on antimicrobial activity of BUA against a wide range of human pathogenic bacteria and fungi. To the best of our knowledge, we have been reporting here the antioxidant activity of BUA for the first time. The above results suggest the possible use of BUA as a potent chemical entity for developing pharmacologically important drugs. The *in vivo* toxicological studies on BUA and its mode of action are being investigated for further exploitation.

Table 2 - Determination of antioxidant activity of BUA.

Samples	DPPH IC_{50} (μg/mL)	β-carotene/linoleic acid inhibition (%) at 1000 μg/mL
BUA	400 ± 3.9	67.8 ± 0.8
Ascorbic acid	38 ± 1.2	92.8 ± 0.8

Methanol was used as negative control.

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Conflict of interest statement

We declare that we have no conflict of interest.

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