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In vitro antioxidant and antimicrobial activity cycloart-23-ene-3 β , 25-diol (B2) isolated from *Pongamia pinnata* (L. Pierre)

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ABSTRACT

Objective: To evaluate the *in-vitro* antioxidant and antimicrobial activity of cycloart-23-ene-3 β , 25-diol (called as B2) isolated from stem bark of *Pongamia pinnata*. **Methods:** *In vitro* antioxidant activity of B2 was determined by methods for determination of DPPH radical scavenging, reducing power, superoxide anion radical scavenging, hydroxyl radical scavenging, hydrogen peroxide scavenging, metal chelating and nitric oxide radical scavenging at the doses of 20, 40, 60, 80 and 100 μ g/mL, respectively. β -tocopherol with same concentration was used as a standard antioxidant. *In vitro* antimicrobial activity of B2 was determined by cup plate method in different concentration range of 10-100 μ g/mL. **Results:** The results indicated that dose dependent % reduction against DPPH radical, reducing power, superoxide anion radical scavenging, hydroxyl radical scavenging, metal chelating, hydrogen peroxide scavenging and nitric oxide radical scavenging by B2 and β -tocopherol. **Conclusions:** It is concluded that cycloart 23-ene-3 β , 25 diol (B2) showed dose dependent antioxidant activity. B2 showed more DPPH radical scavenging, reducing power, superoxide scavenging, hydroxyl radical scavenging, metal chelating scavenging, hydrogen peroxide radical scavenging and nitric oxide radical scavenging activity than β -tocopherol and in case of antimicrobial activity B2 exhibited broad-spectrum activity against bacteria and strong activity against yeast type of fungi.

1. Introduction

There are many kinds of reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radical and singlet oxygen[1]. Free radicals are produced in normal or pathological cell metabolism[2]. Oxidation reactions, often radical initiated, are important processes in biological systems[3]. Antioxidant supplements or antioxidant containing plants may be used to help the human body to reduce oxidative damage[2,4-6]. Many bioactive compounds from plants have been screened for their potential uses as alternatives medicines for the treatment of many infectious diseases and also in preservation of food from the toxic effects of oxidants. In modern days the antioxidants and antimicrobial activities of plant have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy[7]. The trend to use bioactive compounds from plants may act as natural antimicrobial and antioxidants influence the health. Also many bioactive compounds from plants have been used as a source of medicinal agents to cure urinary tract infections, cervicitis vaginitis, gastrointestinal disorders, respiratory diseases, cutaneous affections, helmintic infections, parasitic protozoan diseases and inflammatory processes[8].

Pongamia pinnata (Linn) Pierre [family Fabacae , synonym; *Pongamia glabra* Vent., *Derris indica* (Lam.) Bennet, *Cystisus pinnatus* Lam.] popularly known as 'Karanj' or 'Dittouri' in Hindi and Indian beech, Pongam oil tree, Hongay oil tree in English[9,10]. *Pongamia pinnata* reported significant antihyperglycaemic and antilipidperoxidase[11-14], antifungal and antibacterial[15], antimicrobial[16-18]. Recently, we have reported the antihyperglycaemic activity of alcoholic[19] and petroleum ether extract[20] of *Pongamia pinnata* (L.) and their concomitant administration with synthetic oral hypoglycaemic drugs[21]. We have also reported antihyperglycaemic[22] and antidiabetic[23] activity activity cycloart-23-ene-3 β , 25-diol isolated from stem bark of *Pongamia pinnata* in streptozotocin-nicotinamide induced

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diabetic mice. However, there is paucity of reports on the antioxidant antimicrobial activity of cycloart-23-ene-3 β, 25-diol (B2). Therefore, the objective of present investigation was to evaluate *in vitro* antioxidant and antimicrobial activity of cycloart-23-ene-3 β, 25-diol (called as B2).

2. Material and methods

2.1. Cycloart-23-ene-3 β, 25-diol

Isolation and characterization of cycloart-23-ene-3 β, 25-diol (B2) has been previously reported by Badole and Bodhankar (2009). The structure of cycloart-23-ene-3 β, 25-diol (B2) is shown in Figure 1[22].

2.2. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), α-tocopherol, 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), thiobarbituric acid (TBA), trichloraceticacid (TCA), potassium ferricyanide, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), ferric chloride, ascorbic acid and 2-deoxyribose were purchased from were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium nitroprusside, hydrochloric acid, methanol, sodium hydroxide, and hydrogen peroxide (H_2O_2) were purchased from Merck (India). Disodium hydrogen phosphate (Research Lab, India), Potassium dihydrogen phosphate (S.D. Fine, Mumbai), sulphanilamide (Sisco Research Lab Pvt. Ltd, India), phosphoric acid (S.D. Fine Chem. Ltd. India) and napthylethylenediamine dihydrochloride (Unichem Ltd, India) were purchased from respective vendors. All other chemicals were analytical grade.

2.3. Determination of free radical scavenging activity (DPPH)

The scavenging activity of the B2 was determined by using 1,1-diphenyl-2-pycrylhydrazyl (DPPH) assay previously reported by Bakar *et al*(2009). 1 mL of B2 solution (20, 40, 60, 80 and 100 μg/mL) and α-tocopherol (20, 40, 60, 80 and 100 μg/mL) were mixed with 5.0 mL of 1 mM DPPH in absolute methanol. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The absorbance was read by UV-visible spectrophotometer (Jasco V-530, Japan) against methanol at 517 nm[24]. The experiment was repeated triplicate. The activity was expressed as percentage DPPH-scavenging activity relative to the control, using the following equation:

% inhibition (DPPH) = [1-(absorbance of sample/absorbance of control)] × 100

2.4. Determination of reducing power

Reducing power of B2 was determined by previously reported method Oyaizu (1986). 1 mL B2 solution (20, 40, 60, 80 and 100 μg/mL) as well as α-tocopherol (20, 40, 60, 80 and 100 μg/mL) were mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The reaction mixture was incubated at 50 ℃ for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 7 000 rpm. for 10 min. 2.5 mL solution from the upper layer was mixed with 2.5 mL distilled water and freshly prepared 0.5 mL $FeCl_3$ (0.1%). The absorbance of sample solutions was read by UV-visible spectrophotometer at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power[25].

2.5. Determination of superoxide anion radical scavenging activity

The method described by Liu *et al*(1997) with modification of Oktay (2003) was used for determination of superoxide anion scavenging activity of B2[26,27]. Superoxide radicals are generated non-enzymatically in phenazine methosulphate-nicotinamide adenine dinucleotide (PMS-NADH) systems by the oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0) containing NBT (50 μM) solution and 1 al NADH (78 μM) solution and sample solution of the B2 (20, 40, 60, 80 and 100 μg/mL) as well as α-tocopherol (20, 40, 60, 80 and 100 μg/mL) in methanol. The reaction was initiated by adding 1.0 mL of phenazine methosulphate (PMS) solution (10 μM) to the mixture. The reaction mixture was incubated at 25 ℃ for 5 min, and the absorbance was read at 560 nm by UV-visible spectrophotometer. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity[26,27]. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% inhibition (Superoxide anion) = [1-(absorbance of sample/absorbance of control)] × 100

2.6. Determination of hydroxyl (OH) radical scavenging activity

Deoxyribose method of Halliwell *et al*(1987) was used to determine the hydroxyl radical scavenging activity[28]. The reaction mixture, which contained B2 (20, 40, 60, 80 and 100 μg/mL) as well as α-tocopherol (20, 40, 60, 80 and 100 μg/mL), deoxyribose (3.75 mM), H_2O_2 (1 mM), potassium phosphate buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM) and ascorbic acid (0.1 mM), was incubated in a water bath at (37±0.5) ℃ for 1 h. 1 mL of TBA (1% w/v) and 1 mL of TCA (2.8% w/v) were added to the mixture and heated in a water bath at 100 ℃ for 20 min. The absorbance of the resulting solution was measured UV-visible sprectophotmeter at 532 nm[28]. All the analyses were performed in triplicates. The percent inhibition of deoxyribose degradation was calculated by the following formula:

% inhibition [hydroxyl (OH.) radical] = [1-(absorbance of sample/absorbance of control)] × 100

2.7. Determination of metal chelating scavenging activity

The chelation of ferrous ions by B2 and α-tocopherol were determined by the method of Dinis *et al*(1994)[29]. B2 (20, 40, 60, 80 and 100 μg/mL) as well as α-tocopherol (20,

40, 60, 80 and 100 μg/mL) were added to a 0.05 mL solution of $FeCl_2$ (2 mM). The reaction was initiated by the addition of 0.2 mL ferrozine (5 mM) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was read UV-visible spectrophotometer at 562 nm[29]. All tests and analyses were run in triplicate and averaged. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following formula:

% inhibition (Metal chelating) = [1-(absorbance of sample/absorbance of control)] × 100

2.8. Determination of hydrogen peroxide (H_2O_2) scavenging activity

The ability of the B2 to scavenge H_2O_2 was determined by the method of Ruch *et al* (1989)[30]. Solution of H_2O_2 (4 mM) was prepared in phosphate buffer (pH 7.4). H_2O_2 concentration was determined UV-visible spectrophotometer from absorption at 230 nm in UV-Visible spectrophotometer (Jasco V-530). B2 (20, 40, 60, 80 and 100 μg/mL) as well as α-tocopherol (20, 40, 60, 80 and 100 μg/mL) in methanol was added to a H_2O_2 solution (0.6 mL, 40 mM). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing in phosphate buffer without H_2O_2[30]. The percentage of H_2O_2 scavenging was calculated by following formula:

% inhibition (H_2O_2) = [1-(absorbance of sample/absorbance of control)] × 100

2.9. Determination of nitric oxide radical scavenging activity

The method described by Green *et al*(1982) was used for determination of nitric oxide radical scavenging of B2[31]. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3.0 mL of B2 (20, 40, 60, 80 and 100 μg/mL) as well as α-tocopherol (20, 40, 60, 80 and 100 μg/mL) dissolved in the methanol and incubated at 25 ℃ for 150 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read by UV-visible spectrophotometer at 546 nm[31]. The percentage inhibition of nitric oxide was calculated by the following formula:

NO scavenged (%) = [1-(absorbance of sample/absorbance of control)] × 100

2.10. Determination of antimicrobial activity by cup plate method

Antimicrobial activity of B2 was screened against different test organisms. The particular concentration 10-100 μg/mL of B2 were prepared and added in each well. Bacterial cultures were grown overnight in sterile nutrient broth and fungal culture grown for 18-24 h in sterile SDA medium. Their optical density (OD) was adjusted to 0.1. This adjusted OD culture was spread (100 μL) on sterile nutrients agar plates and fungal culture on sterile sabouraud's dextrose agar plate. The cavities were prepared in agar plate by using cork borer and 100 μL of drug solution was added. The plates were incubated at 37 ℃ for 48 h for detection of antibacterial activity and at 28 ℃ for 72 h for the detection of antifungal activity. Zone of inhibition around the drug was recorded in mm. This activity was compared with standard antibiotic tetracycline (bacteria) and fluconazol (antifungal). The test bacteria included were *Staphylococcus aureus* (NCIM2079), *Escherichia coli* (NCIM2345), *Bacillus subtilis* (NCIM2063) and the test fungi included *Aspergillus niger* (NCIM529), *Aspergillus fumigates* (NCIM623), *Candida albicans* (NCIM3471), *Penicillium* (NCIM745) species.

2.11. Statistical analysis

All analyses were performed in triplicate. Data was expressed as mean±SEM. Statistical analysis was carried out by one way ANOVA followed by *post hoc* Tukey test performed using GraphPad InStat version 3.00 for Windows VistaTM BASIC, GraphPad Software, San Diego, California, USA. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Determination of free radical scavenging activity (DPPH)

The DPPH scavenging effect of B2 were (40.44±1.40)%, (43.58±1.21)%, (45.56±0.77)%, (50.91±0.95)%, and (53.06±0.92)% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively. While DPPH scavenging effect of α-tocopherol were (28.86±0.32)%, (30.96±0.99)%, (33.60±1.29)%, (36.38±1.08)% and (42.09±0.75)% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively. The results thus indicated that significant ($P<0.001$) decrease in the DPPH radicals may be due to the scavenging ability of B2 and α-tocopherol. Free radical scavenging activity was increased with an increasing concentration. B2 was more effective than α-tocopherol as scavenging compound.

3.2. Determination of reducing power

The reducing power of B2 and α-tocopherol was dose dependent. The absorbance was 0.13±0.01, 0.17±0.01, 0.20±0.01, 0.25±0.01 and 0.27±0.01 at the concentration 20, 40, 60, 80 and 100 μg/mL of B2 respectively. The absorbance by α-tocopherol was 0.05±0.01, 0.07±0.01, 0.09±0.01, 0.11±0.02 and 0.15±0.002 at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively. B2 thus exhibited strong reducing power compared to α-tocopherol.

3.3. Determination of superoxide scavenging

B2 significant inhibited superoxide radicals in a dose dependent($P<0.001$). The percentage (34.67±0.76)%, (40.08±0.69)%, (50.90±0.72)%, (58.94±0.12)% and (63.86±0.41)% inhibition of superoxide radical generation by B2 was at 20, 40, 60, 80 and 100 μg/mL whereas α-tocopherol had (23.95±0.43)%, (33.63±0.43)%, (41.19±0.21)%, (48.87±0.27)% and (57.66±0.59)% inhibition by the at concentration 20, 40, 60,

80 and 100 μg/mL, respectively.

3.4. Hydroxyl (OH) radical scavenging activity

B2 and α-tocopherol significantly (P<0.001) inhibited hydroxyl radical scavenging activity in a dose dependent manner. The hydroxyl radical scavenging activity of B2 was (22.35±0.48)%, (29.77±0.19)%, (43.44±0.56)%, (51.22±0.98)% and (59.88±0.38)% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively. While hydroxyl radical scavenging activity of α-tocopherol was (16.17±0.29)%, (24.05±0.25)%, (30.41±0.64)%, (35.57±0.77)% and (45.63±0.35)% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively.

3.5. Determination of metal chelating scavenging activity

A dose dependent decrease in the absorbance of Fe^{2+}-ferrozine complex was produced by B2 and α-tocopherol. The percentage of metal chelating capacity of 20, 40, 60, 80 and 100 μg/mL of B2 was 26.04±0.75) %, (38.18±0.92)%, (47.61±0.45)%, (52.50±0.53)% and (59.43±1.09)%, respectively. While metal chelating capacity of 20, 40, 60, 80 and 100 μg/mL of α-tocopherol was (12.78±1.18)%, (22.41±0.63)%, (36.41±0.79)%, (43.01±0.63)% and (53.86±0.70)%, respectively.

3.6. Hydrogen peroxide (H_2O_2) radical scavenging activity

B2 and α-tocopherol significantly (P<0.001) inhibited hydrogen peroxide radical scavenging activity in a dose dependent manner. The hydrogen peroxide radical scavenging activity of B2 was (23.27±0.99)%, (31.35±1.40)%, (39.40±0.71)%, (47.14±0.71)% and (51.58±0.41)% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively. While hydrogen peroxide scavenging activity of α-tocopherol was (13.45±0.62)%, (19.93±0.62)%, (24.32±0.76)%, (29.58v0.69)% and (33.33±0.31)% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively.

3.7. Determination of nitric oxide radical scavenging activity

Both B2 and α-tocopherol significantly inhibited nitric oxide radical scavenging activity in a dose dependent manner(P <0.001). Nitric oxide radical scavenging activity of B2 was 22.40%, 34.20%, 42.22%, 52.09% and 58.19% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively. While nitric oxide scavenging activity of α-tocopherol was 17.64%, 26.03%, 35.70%, 42.55% and 51.83% at the concentration of 20, 40, 60, 80 and 100 μg/mL, respectively.

3.8. Determination of antimicrobial activity by cup plate method

Different concentrations of B2 from 10 μg/mL to 100 μg/mL showed linear activity with increase in concentration. This data indicated that the species exhibited broad-spectrum activity against bacteria and strong activity against yeast type of fungi, whereas does not shown activity against *Pseudomonas aeruginosa*, very low activity against mold type of fungi such as *Aspergillus niger*, *Aspergillus fumigatus* and no activity against *Penicillium notatum*. The minimum inhibitory concentration (MIC) of B2 was found to be at 80 μg/mL for bacteria and 100 μg/mL for fungi.

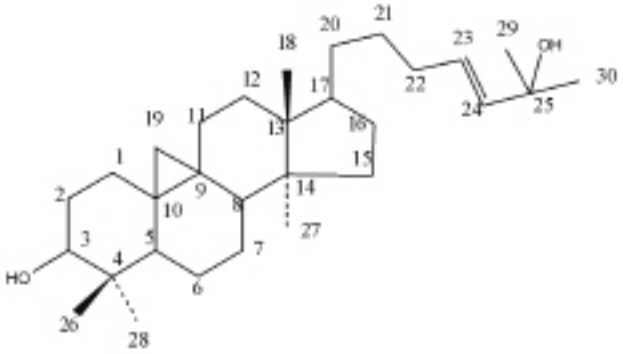

Figure 1. Structure of Cycloart-23-ene-3 β , 25-diol (B2).

4. Discussion

DPPH assay has been widely used to provide basic information on the antioxidant ability of extracts from plant, food material or on single compounds, because this method has shown to be rapid and simple available[32]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability[33,34] and is a useful reagent for investigating the free radical scavenging activities of compounds[35]. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[36]. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and the radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants[37,38].

These results indicated that B2 has a noticeable effect of scavenging free radicals. It was reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic condition[39]. B2 react with free radicals which are the major initiator of the autoxidation chain of fat, thereby terminating the chain reaction[40,41]. It is thus apparent that B2 is free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. B2 had comparably less DPPH radical scavenging activity than α-tocopherol.

Several methods have been developed to measure the efficiency of antioxidants as pure compounds. Different studies have indicated that the antioxidant effect is related to the development of reductones. Reductones were reported to be terminators of free radical chain reactions[42].

The reducing power assay measures the electron-donating ability of antioxidants using potassium ferricyanide reduction method. Antioxidants reduce the ferric ion/ferricyanide complex to the ferrous form, the Perl's Prussian

blue complex[43,44]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[45]. The antioxidant activity of an B2 and α-tocopherol have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[46-48]. The reducing capacity of a B2 and α-tocopherol indicate their potential antioxidant activity.

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals[49,50]. In PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture[51]. B2 had strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than the α-tocopherol.

The binding of ferrous ions by B2 was estimated by the method of Dinis *et al*(1994)[29]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator[52]. B2 and α-tocopherol interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Iron can stimulate lipid peroxidation by Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation[38,53,54]. The data revealed that B2 and α-tocopherol marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity. Metal chelating activity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation[37]. It has been reported that chelating agents which form α bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion[41]. As shown in this study, the formations of the Fe^{2+}-ferrozine complex were prevented by B2 and α-tocopherol.

OH radical is the most reactive free radical in biological systems and it can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions, such as copper and iron. Hydroxyl radical has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. For example, OH^- radicals react with lipid, polypeptides, proteins and DNA, especially thiamine and guanosine. This radical has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity[55]. The highly reactive OH radicals can cause oxidative damage to DNA, lipids and proteins[56]. As is the case for many other free radicals, OH^- radicals can be neutralised if it is provided with a hydrogen atom. The results indicate that B2 had strong hydroxyl radical scavenging activity than α-tocopherol.

The ability of B2 and α-tocopherol to scavenge H_2O_2 was determined according to the method of Ruch *et al*(1989)[30]. H_2O_2 is highly important because of its ability of penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells[28]. Thus, removing H_2O_2 is very important for the protection of living systems. The results indicate that B2 had strong hydrogen peroxide radical scavenging activity than α-tocopherol.

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by B2 and α-tocopherol. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide[57,58] which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide[31]. B2 had comparably more nitric oxide radical scavenging activity than α-tocopherol.

Antimicrobial activity of B2 was screened against different microbial species by using cup plate technique. Antimicrobial activity was checked and results were expressed in terms of zone of inhibition (in mm). Different bacterial and fungal strains were used. B2 showed strong activity against bacteria like *Bacillus subtilis* (MIC- 30 mm), *Staphylococcus aureus* (MIC- 25 mm), *Escherichia coli* (MIC- 22 mm) and fungi as *Candida albicans* (MIC- 34 mm) species. Since many plant terpenoids previously have been found to be responsible for several biological properties, including antimicrobial properties[59-77], hence the antimicrobial activity of B2 would be related to its terpenoid nature of the compounds.

In conclusion, cycloart 23-ene-3 β, 25 diol (B2) isolated from stem bark of *Pongamia pinnata* show dose dependent antioxidant activity. B2 show comparatively less DPPH radical scavenging activity but more reducing power, superoxide scavenging, hydroxyl radical scavenging, metal chelating scavenging, hydrogen peroxide radical scavenging and nitric oxide radical scavenging activity than α-tocopherol and B2 exhibits broad-spectrum activity against bacteria and strong activity against yeast type of fungi. The results confirm antioxidant and antimicrobial activity of B2.

Conflict of interest statement

We declare that we have no conflict of interest.

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