INTRODUCTION

Natural diet is rich sources of active compounds polyphenols, carotenoids, flavonoids, vitamins, proteins, polysaccharides, and certain important trace metals such as zinc and selenium [1-3]. These play a major role as an antioxidant source in reduce cellular damages [4]. Antioxidants are man-made or natural substances, diet high in green leafy vegetables and fruits, which are good sources of antioxidants, have been found to be healthy [5]. Antioxidant substances could be natural or synthetic. Natural antioxidants are obtained from natural sources are safe, non-toxic, inexpensive, and have been used in food, cosmetics, and other related industries. On the other hand, synthetic antioxidants are substances created from chemical process; in excessive usage, they are reported as toxic [6]. Hence, day by day, researchers are in the search of new source of natural antioxidants.

The plant Agathi (Sesbania grandiflora) belongs to the family Fabaceae of genus Sesbania and species Grandiflora. It is widely grown in India, Indonesia, Myanmar, the Philippines, Thailand, and South-East Asian countries. The leaves are bitter rich in Vitamin C, calcium, sterols, saponin, quercetin, myricetin, and other chemical antioxidants [7]. It is reported that, the seeds of S. grandiflora rich with natural antioxidant Vitamin E and Phytocerol contents. Hence, herein we made an attempt to find the DNA sugar protectant and non-toxic nature of antioxidant content of ethanol-water extract of S. grandiflora [8].

METHODS

Agathi seeds were obtained from authentic source, Bangalore Karnataka state, India. 100 g of well dried Agathi seeds were grounded into a fine powder using a domestic electric grinder.

RESULTS

The different extracts (water, alcohol; alcohol; water; and hexane) of Agathi seeds (Sesbania grandiflora Linn) were evaluated using various antioxidant and other relevant assays like DNA sugar protection and antioxidant activities.

Conclusion:

These results establish the antioxidant potential of the extract, which could be used as natural antioxidant source.

Keywords: DNA, Lymphocytes, Fenton, Erythrocytes, Agathi, Antioxidants.
(0-25 μL) were made up to 1 mL with distilled water. To this 1 mL of 5% phenol and 5 mL of concentrated sulphuric acid were added keeping the mixture ice-cold water bath. Orangeish color developed was read at 520 nm immediately. The sugar concentration of the extract was calculated according to the standard glucose calibration curve.

Determination of total phenol content
The total phenolic content of the agathi ethanol-water extract was determined according to the method of Folin-Ciocalteu reaction [11] with appropriate modifications, using gallic acid as standard. An aliquot of the samples (10-40 μL) was mixed with 50% Folin-Ciocalteu reagent; the volume was made up to 1 mL with methanol:water mixture (50:50 v/v). Further, the mixture was then allowed to stand for 1 min at room temperature followed by the addition of 20% Na₂CO₃. Further, the absorbance was measured at 725 nm. Results were expressed as milligrams of gallic acid equivalents per gram.

Antioxidant activity
1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity
DPPH radical scavenging activity was assessed as per the standard method [12]. DPPH, α-tocopherol, and Ascorbic acid were procured from Sigma (St. Louis, USA). Different extracts of Agathi seeds at various concentrations ranging from 10 to 100 μg were mixed in 1 mL of freshly prepared 0.5 mM DPPH ethanolic solution and 2 mL of 0.1 M acetate buffer at pH 5.5. The resulting solutions were then incubated at 37°C for 30 min and measured colorimetrically at 517 nm in a Shimadzu ultraviolet (UV)-1601 spectrophotometer (Tokyo, Japan). Standard antioxidants such as α-tocopherol and Ascorbic acid, all at 400 μM and 100 μM respectively, were used as positive controls under the same assay conditions. Negative control was without any inhibitor or extract. Lower absorbance at 517 nm represents higher DPPH scavenging activity. Percent DPPH radical scavenging activity of the extracts was calculated accordingly from the decrease in absorbance at 517 nm in comparison with the negative control.

% Viability = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100

Hydroxyl radical scavenging activity
The hydroxyl radical scavenging activity was determined using Deoxyribose assay [13] with some modifications. The reaction mixture contained FeCl₃ and ascorbate (100 μM), H₂O₂ (1 mM), EDTA (100 μM), 2-deoxy-D-ribose (2.8 mM), and 1 mL of 0.1 M potassium phosphate buffer (pH 7.4) mixed in various concentrations of Agathi seeds extract (50–400 μg/mL). The reaction mixture was incubated for 1 h at 37°C. The reaction was terminated by adding 1 mL each of Trichloroacetic acid (2.8%) and Thiobarbituric acid (0.5%); this mixture was placed in boiling water bath for 15 min. After cooling, the reaction mixture was centrifuged for 5 min at 5000 rpm. The control was without any test compound and the readings were taken at 535 nm. The percentage hydroxyl radical scavenging activity was determined by comparing with control. Decreased absorbance of the reaction mixture indicated decreased oxidation. Consider the following:

% inhibition = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{test}}{\text{Absorbance}_{control}} \times 100

Determination of antioxidant activity using erythrocyte ghost and linolenic acid micelles
The erythrocyte membranes (ghosts) preparation was carried out as per the standard procedure [14]. In brief, fresh venous human blood samples were drawn with ACD anticoagulant (acid citrate dextrose) mixed well and refrigerated centrifuged, the obtained pellet was washed 3 to 5 times using isotonic phosphate buffer saline (PBS 5 mM, pH 7.4, and 150 mM NaCl). Further, the cell pellet was suspended in hypotonic (PBS 5 mM, pH 7.4 at 4°C) for hemolysis to take place. Further, contents were refrigerated centrifuged at 12,000 rpm for 20 min. The obtained erythrocytes were separated from plasma and buffy coat again washed with fresh hypotonic phosphate buffer and centrifuged at 1500 rpm to remove unlysed RBC cells. The membranes were dispersed pale yellowish pink “ghost” suspended in isotonic 5 mM phosphate buffer, pH 7.4. By Bradford’s method, the protein content of ghost was estimated [6]. Ghost suspension (200 μg) and linolenic acid (1.8 μg) were subjected to peroxidation by Fenton reactants (ferrous sulfate and ascorbic acid) (10:100 μM) in a final volume of 0.5 mL Tris-buffered saline (TBS 100 mM, pH 7.4, and 0.15 M NaCl) with increasing concentration of agathi seeds extract (10 to 50 μg); the contents were incubated for an hour and 1% TBA was added. Finally, the contents were kept in a boiling water bath for 15 min and then cooled, centrifuged to remove precipitate if any. The color developed was read at 535 nm using UV visible spectrophotometer.

% inhibition = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{test}}{\text{Absorbance}_{control}} \times 100

Isolation of lymphocyte and their protection
Human peripheral lymphocytes were isolated according to the standard protocol [15]. Fresh venous blood (10 mL) mixed with four volumes of hemolyzing buffer (150 mM NH₄Cl in 10 mM tris buffer, pH 7.4) and mixed well. The contents were incubated for 30 min at 4°C and centrifuged at 1200 rpm for 20 min and the supernatant was discarded. The pellet was washed twice to thrice with 10 mL of 250 mM m-insotlin in 10 mM phosphate buffer, pH 7.4, and re-suspended in the same solution. The cell viability was determined by trypan blue exclusion method. Percentage viability was calculated as follows:

% Viability = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100

Determination of DNA sugar damage by spectrophotometric method
Fenton’s reactants induced oxidative DNA sugar damage was determined according to the standard protocol [16]. In brief, the reaction mixture in a total volume of 1 mL containing 1 mg calf thymus DNA was treated with Fe²⁺ (10 mM), EDTA (10 mM) and H₂O₂ (2 mM) without or with various concentrations of the extract (10–50 μg) in potassium phosphate buffer (20 mM, pH 7.4). Ascorbic acid (10 mM) was added to the reaction mixture and was incubated at 37°C for 1 h in water bath with shaker. To 1 mL of the above mixture 1 mL of Trichloroacetic acid and 1 mL of 1% TBA were added and boiled for 20 min. The contents were cooled and the pink color absorbance was read spectrophotometrically at 523 nm.

% inhibition = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{test}}{\text{Absorbance}_{control}} \times 100

Statistical analysis
Statistical analysis was done in SPSS (Windows Version 10.0.1 Software Inc., New York) using a one-sided student’s t-test. All results refer to mean±SD. p<0.05 was considered as statistically significant when compared to relevant controls.

Table 1: Cytotoxicity of ethanol-water extract of agathi (Sesbania grandiflora Linn) seeds toward human blood lymphocytes

| Concentration (mg/mL) | Viability (%) |
|----------------------|--------------|
| No treatment         | 97.0±3.21    |
| Fenton reactant alone| 35.0±0.02    |
| Fenton reactant+0.2 (extract) | 94.0±1.23    |
| Fenton reactant+0.4 (extract) | 95.0±1.24    |
| Fenton reactant+0.6 (extract) | 96.0±1.77    |
| Fenton reactant+0.8 (extract) | 96.0±1.78    |

Data are expressed as the mean standard deviation (n=3). Means with different letters (a–b) are significantly different (p<0.05)
RESULTS AND DISCUSSION

The different extracts of Agathi seeds were subjected to proximate analysis and DPPH radical scavenging activity. The proximate analysis showed that the ethanol-water extract contains Polyphenols, proteins, and negligible amount of sugars (data not shown). The antioxidant and DNA protective nature and non-toxic nature to cells study results are as follows.

**DPPH radical scavenging effect**

The DPPH radical scavenging activity of ethanol-water extract of Agathi seeds on Fe³⁺ dependent hydroxyl radical generation was reported and confirmed using a direct approach [17,18]. The results obtained are shown in Table 2, the ethanol-water extract of Agathi seeds exhibited powerful DPPH radical scavenging activity of 88% at 25 µg, which was much more than water, ethanol, hexane extract (at 100 µg dosage each), which showed DPPH radical scavenging activity of 40%, 41%, 51%, and 46%, respectively. The well-known antioxidants such as alpha-tocopherol (85.5 µg) and ascorbic acid (100 µg) showed 82% and 78% DPPH radical scavenging activity respectively. The results indicate that ethanol-water extract is a powerful free radical scavenger compared to other extracts and known antioxidants. Hence, for further studies only ethanol-water extraction of Agathi seeds considered.

**Hydroxyl radical scavenging activity**

The short-lived hydroxyl radicals are highly reactive of all the reduced form of dioxygen and in excess, initiate cell damage in *in vivo* model [19,20]. The antioxidant effect of Agathi seeds extracts on hydroxyl radicals generated by Fe³⁺ ions was measured by the extent of deoxyribose degradation, which is an indicator of TBA–MDA adducts formation. Among the various extracts tested (Fig. 1). The ethanol-water extract showed maximum hydroxyl radical scavenging activity by 69% at 50 µg which is comparatively good when compared to standard antioxidant Ascorbic acid (400 µM) and alpha-tocopherol (400 µM) which were 75% and 71% respectively. This implies that ethanol-water extract could be an effective hydroxyl radical scavenger.

**Inhibitory effect of AWE on fenton reactant-induced DNA sugar damage**

To find the chelating and the inhibitory effect of ethanol-water extract of Agathi seeds against iron-dependent oxidation of calf thymus DNA sugar was tested by a TBA–MDA assay. As shown in Fig. 2, the extract offered effective inhibition by 55% at 50 µg against ferrous sulfate: ascorbate-induced [16], DNA sugar damage using erythrocyte ghost as a source of lipids, when compared to Ascorbic acid (400 µM) and Alpha-tocopherol (400 µM), showed 62% and 71% respectively. When Linolenic acid micelle was used as a source of lipids, the maximum protection provided by the ethanol-water extract is 55% when compared to Ascorbic acid (400 µM) and Alpha-tocopherol (400 µM), showed 66% and 71%, respectively. Similar studies have reported that extracts of Curry leaves, Turmeric, *Coles aromatics* leaves extract exhibit chelating effect of ferrous ions and a reducing capacity [16,19,21,22].

**Table 2: Diphenyl-2-picrylhydrazyl radical scavenging activity of ethanol-water extract of Agathi (Sesbania grandiflora Linn) seeds**

| Extraction          | Concentration (µg/mL) | Percentage DPPH radical scavenging activity |
|---------------------|-----------------------|--------------------------------------------|
| Negative control    | No                    | 0                                          |
| Water               | 100                   | 40±1.2                                     |
| Ethanol             | 100                   | 51±2.0                                     |
| Hexane              | 100                   | 46±2.2                                     |
| Ethanol-water       | 25                    | 88±1.7                                     |
| Alpha-tocopherol    | 85.5 (400 µM)         | 82±1.5                                     |
| Ascorbic acid       | 100                   | 78±2.2                                     |

Each value is expressed as mean±standard deviation (n=3). DPPH, 1,1-dephenyl-2-picrylhydrazyl (DPPH) radical. DPPH Diphenyl-2-picrylhydrazyl.
AUTHORS’ CONTRIBUTIONS
We hereby declare that all the authors contributed equally in preparing and finalizing this review manuscript.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

FUNDING
This study was done without any external financial support.

REFERENCES
1. Dinesha R, Kumar NS, Chikkanna D, Joshi V. Metal ion chelation and anti-glycation properties of polysaccharides of *Phyllanthus amarus* plant. GSC Biol Pharm Sci 2021;15:349-53.
2. Dinesha R, Chikkanna DS, Maheshwara KV, Vedamurthy J. Anti-glycation and antioxidant properties of *Abutilon indicum* plant leaves. Int J Sci Res Arch 2021;2:269-73.
3. Rao BN. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asia Pac J Clin Nutr 2003;12:9-22.
4. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.
5. Anuj Y, Rewa K, Mishra JP, Seweta S, Shashi P. Antioxidants and its functions in human body-a review. Res Environ Life Sci 2016;9:1328-31.
6. Kornienko JS, Smirnova IS, Pugovkina NA, Ivanova JS, Shilina MA, Grinchuk TM, et al. High doses of synthetic antioxidants induce premature senescence in cultivated mesenchymal stem cells. Sci Rep 2019;9:1296.
7. Zarena AS, Shubha G, Vineth R. Antioxidant, antibacterial, and cytoprotective activity of Agathi leaf protein. J Anal Methods Chem 2014;2014:989543.
8. Huma S, Ghazala HR, Muhammad Z, Shakeel A, Hina Z. Tocopherol and phytosterol profile of *Sesbania grandiflora* (Linn.) seed oil. J Med Plants Res 2012;6:3478-81.
9. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 1976;72:248-54.
10. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956;28:350-6.
11. Dinesha R, Leela S. Antioxidant effects of 28-kda Protein from Turmeric (*Curcuma longa* L.). Asian J Pharm Clin Res 2011;4:75-9.
12. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945-8.
13. Chung SK, Osawa T, Kawakishi S. Hydroxyl radical scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). Biosci Biotechnol Biochem 1997;61:118-23.
14. Dodge JT, Mitchell C, Hanahan DJ. The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch Biochem Biophys 1963;100:119-30.
15. Smitha S, Dhananjaya BL, Dinesha R, Leela S. Purification and characterization of a approximately 34-KDa antioxidant protein (beta-turmerin) from turmeric (*Curcuma longa*) waste grits. Biochemie 2009;91:1156-62.
16. Cao W, Chen WJ, Zheng XH, Zheng JB. Modified method to evaluate the protection of the antioxidants against hydroxyl radical-mediated DNA damage. Acta Nutrimenta Sin 2008;30:74-7.
17. Biba V, Akhil BS, Romani P, Sujaathan K. Free radical scavenging properties of Ammona squamosa. Asian Pac J Cancer Prev 2017;18:2725-31.
18. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. J Agric Food Chem 2001;49:3420-4.
19. Mylarappa BN, Ramadas D, Leela S. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf extract (*Murraya koenigii* L.). Food Chem 2008;106:720-8.
20. Rafael R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proc Natl Acad Sci USA 2018;115:5839-48.
21. Kumar NS, Aliya N, Vedamurthy J, Dinesha R. Protective effect of Coleus aromaticus plant protein against t-BOOH induced cell damage. Int J Pharm Biol Sci 2021;12:112-9.
22. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci 2014;2:53.