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ORIGINAL RESEARCH ARTICLE

Comparative Study on Antioxidant Activities of Black and White Seed Varieties of Cow –Hedge *(Mucuna pruriens* L.*)*

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ABSTRACT

Objectives: To investigate the antioxidant activity of methanolic seed extract of two varieties of *Mucuna pruriens.*

Methods: Invitro antioxidant activity of methanolic seed extract of *M . pruriens* was evaluated by DPPH, total phenol, flavanoid , anthocyanin superoxide radicals scavenging and metal chelation activity.

Results: 1, 1-diphenyl 2-picryl hydrazyl (DPPH) assay, total phenol and total flavanoids of black seed were higher than the white seed. On the other hand superoxide scavenging, metal chelates activity of the black seed variety also higher than white seed. This study showed that the medicinal potential of black and white seed varieties of *M. pruriens* and positive relationship between total phenol content and antioxidant activities in *M. pruriens*.

Conclusion: The results of the present study strongly revel that the methanolic extract of black seeds of *Mucuna pruriens* has potent antioxidant activity than the white seed extracts.

Key words: Antioxidant activity, *Mucuna pruriens*, Cow-hedge,DPPH.

1. INTRODUCTION

Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other bio molecules and thus, prevent or repair the damage of the body cells that is caused by oxygen [28,30]. Apart from their role as health benefactors, antioxidants are also added to food to prevent or delay its oxidation, normally initiated by free radicals formed during the food's exposure to environmental factors such as air, light and temperature. Antioxidants are important in the prevention of human diseases, as it functions as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [3]. Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds, possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and aging [13,20]. Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic, therefore, the importance of search for natural antioxidants has greatly increased in the recent years [11]. Plants are a potential source of natural antioxidants or phytochemical antioxidants are secondary metabolites of plants such as Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, *etc*. are among the antioxidants produced by plants for their own sustenance. The therapeutic benefits of medicinal plants are usually contributed to their antioxidant properties. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants [19].*Cow-hedge (Mucuna pruriens) is* one of the potential tropical legume having good nutritional qualities and medicinal properties. It belongs to the family Fabaceae and is distributed in Southern

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and Southeastern Asian regions. *M. pruriens* beans have also been found to contain high amounts of protein and carbohydrate and to be a rich source of macro- and microelements. In addition to the nutritive value of the seeds, as cited [29] different preparations of them are used for the management of several free radical-mediated diseases, such as rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders in the Ayurvedic system of medicine. Many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties. The present aim of our research is to study the antioxidant activity of methanolic extract of black and white seed varieties of *M.pruriens.*

2. MATERIALS AND METHODS

2.1. Plant material and Extraction

Mucuna pruriens fresh seeds (black and white) were harvested from the Botanical garden of Annamalai University, Annamalai Nager (**Fig 1**). The seeds were dried in shade and powdered in a mechanical grinder. The powder was extracted by 1000 ml of methanol by using a Soxhlet extractor for 72 h at temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatmann filter paper (No. 1) and then concentrated in vacuum and dried. The extract thus obtained was directly used in biochemical assay**.**

Fig 1: Cow –hedge pods

2.2. Biochemical assay

2.2.1. Proximate analysis

The total moisture, ash, fat, crude fibre, protein and carbohydrate contents different varieties of cow hedge studied were estimated by the standard procedure of the AOAC [1].

2.2.2. Total anthocyanin measurement

Total anthocyanins were measured according to a modification of the method described [8,13].The concentration (mg/L) of each anthocyanin was calculated according to the following formula and expressed as Cyanidin-3-glucoside(Cy-3-glc) equivalents:Concentration (mg/L) of each anthocyanins to calculate the concentration of each anthocyanins and thus result reported is expressed as Cy-3-glc equivalents.

2.2.3. Total phenolic acid assay

The total phenolic acid assay was conducted as described [17]. Total phenolic acid content assay was carried out using Folin-Ciocalteu agent. The total phenolic acid content was expressed as mg Gallic acid equivalents (GAE)/g samples.

2.2.4. Total flavonoid assay

The total flavonoid assay was conducted according to the method [17]. Total flavonoids assay was conducted using Aluminium Chloride Colorimetric method Total flavonoids content was expressed as mg Catechin equivalents (CE)/g samples.

2.2.5. DPPH radical scavenging activity

Free radical scavenging activity of the *M. pruriens* extracts were determined by using a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) [4]. The percentage of scavenging activity was calculated as: Ac - As /Ac ×100 where 'Ac' is the absorbance of control (without extract) and 'As' is the absorbance of sample. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC_{50} value. IC_{50} is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system. The IC_{50} values are inversely proportional to the antioxidant activity.

2.2.6. Superoxide radical scavenging activity

Superoxide radical scavenging activity study was performed according to the method [16]. Percentage radical scavenging activity (RSA) was calculated using the formula: RSA% = OD of control - (OD of sample - OD of sample control) / OD of control.

2.2.7. Metal chelating activity

The chelation of ferrous ions by the extract was estimated by the method [5] with slight modification and compared with that of EDTA, BHT and that of ascorbic acid. The percentage inhibition of ferrous–ferrozine complex formation was calculated using the formula: Percentage of chelation = (Ac – As /Ac × 100 where 'Ac' is the

absorbance of control, 'As' is the absorbance of sample.

2.3. Statistical analyses

Data were expressed as mean ± standard deviation from triplicate determination.

3. RESULTS

3.1. Proximate analysis of raw materials and dry weight of the extracts

The chemical composition of two varieties of *M. pruriens* (Cow-hedge) is shown in (**Table 1**).

3.2. The total anthocyanin measurement

Anthocyanin is one of the important secondary metabolities which helps for the antioxidant activities. Table 3 shows the anthocyanine content of two varieties of *M.pruriens*. Methanolic, extract of black seeds possessed markedly higher anthocyanin content 8.71±0.10 mg/g. than white seed extract 0.56±0.78.

3.3. Total phenol content

Mostly antioxidant activities of plant sources are due to the presence of phenolic-type compounds. Due to their hydroxyl groups, phenols are very important plant constituents with scavenging ability. The antioxidant activity has correlation with total phenolic content.The total phenol content of cow-hedge was reported as gallic acid equivalent concentration (mg/ml). The result showed that cow-hedge contained a mixture of phenolic compounds. Table 3 shows total phenolic content (TPC) of two varieties. The total phenolic content (TPC) was markedly higher in black seed extracts (8.71±0.10 mg/mL) than the white seed (7.02±0.63).

3.4. Total flavanoid content

The biological properties, including cytotoxic and antioxidant properties, of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds. Table 3 shows the flavonoid content of two varieties of *M. pruriens*. Methanolic extract of black seeds possessed markedly higher flavonoid content of black seed 3.0±0.54 mg/g than white seed (2.19±0.01mg/g) extracts.

3.5. DPPH radical scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples. (**Table 2**) shows the scavenging activity of different varieties of Cow-hedge, on DPPH radicals of various concentrations. The scavenging activity of cow-hedge on DPPH radicals increased with increasing concentration (50-250 µg/mL). IC_{50} value (the amount of antioxidant material required to scavenge 50% of free radical in the assay system of standard was observed at 207.5 µg/mL). There was inverse relationship between IC_{50} value and antioxidant activity. The results indicate the methanolic extract of black seed possess highest DPPH activity (235.03±1.2 µg/mL) than the white seed extracts.

3.6. Superoxide radical scavenging activity

Superoxide anion is a highly toxic species and generated by different biological reactions in the physiological system. In the present study, the decrease in absorbance at 590 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The increase in percentage inhibition of superoxide radical generation with increasing in concentration of the extract and standard compounds (Quercetin). The plant extract showed good superoxide radical scavenging activity. Based on the IC_{50} values of the results obtained as represent in Table 2, it is clear the radical scavenging activity in black seed (380.0±0.9 µg/mL) than white seed (409.0±0.2 µg/mL).

3.7. Metal chelating activity

The method of determination of metal chelating ability is based on chelation of Fe^{2+} ions by the ferrozine reagent [29]. A complex with Fe^{2+} ions is formed in the reaction which gives absorbance. This formation of the complex is disturbed in the presence of other agents with metal chelating property and absorbance decreases with the reduction of formation of red colored complex. Measurement of the rate of reduction of the color, therefore allows estimation of the chelating activity of the co-existing chelator. In this present experimental setup, the formation of ferrous complex with ferrozine reagent was interfered by both extract and standard compounds (EDTA). Metal chelating power of two varieties of cow-hedge shown in (**Table 3**). The IC50 values were found to be the black seed was higher (108.8±3.6 µg/mL) than the white seed extracts (129.47±2.4 µg/mL)

Table 1: Proximate composition of different varieties of cow-hedge

Parameters	Black seed	White seed
Moisture (%)	9.41±0.80	8.91±0.65
Ash (%)	0.53±0.09	0.31±0.74
Carbohydrate (%)	5.72±0.91	4.0±0.01
Fats (%)	6.77±0.05	6.07±0.98
Protein	23.14±0.38	19.72±0.42
Crude fibre (%)	0.87±0.04	0.62±0.01

Table 2: IC $_{50}$ Value of DPPH Scavenging, superoxide scavenging and metal chelating activities of different varieties of cow-hedge

Parameters	Black seed	White seed
DPPH	235.03±1.2	297.1±7.4
Superoxide scavenging	380.0±0.9	409.0±0.2
Metal chelating	108.8±3.6	129.47±2.4

Table 3: Total phenolic content(TPC), flavanoid content and anthocyanin content of different varieties of cow-hedge.

Parameters	Black seed	White seed
TPC mg/g (%)	8.71±0.10	7.02±0.63
Anthocyanine mg/g	0.756±0.04	0.56±0.78
Flavanoids mg/g	3.0±0.54	2.19±0.01

4. DISCUSSION

Antioxidants are compounds which can protect the human from oxidative agents through different mechanisms [6,26] .Natural antioxidants that are present in herbs and species are responsible for inhibiting or preventing the deleterious consequence of oxidative stress. Species and herbs contain free radical scavengers like polyphenols, flavanoids and phenolic compounds. Natural antioxidants are preferred in allopathic drugs to overcome the side effects. Most of the polar compound such as phenolic and flavanoid substance is potent inhibitor of reactive oxygen species attack.

The present study demonstrates the antioxidant activity of methanolic extract of black and white seeds of *M.pruriens*. Antioxidant compounds isolates and used as a compound for prevention and treatment of free- radical mediated disorder. [20] polyphenolic compound such as phenolic group widely distributed in plant which have been reported to multiple biological effect, including antioxidant, free radical scavenging abilities, anti inflametry [26].

The screening of the black and white seeds of *M.pruriens* indicate that the presence of high phenolic compound may be due to presence of tannin and flavanoids the high content of total phenols in plants is held responsible for the better antioxidant properties [27]. The highest activity of total phenolic content of *M. pruriens* seeds extracts 8.71±0.10 mg/mL. This finding in the agreement with some previous studies which reported the total phenolic content of *M.pruriens* was higher than the other part of the plant *Beta vulgaris*, *Petroselinum crispum*, *corianderum sativum* [23] cantaloupe species [9]. The plant which is high antioxidant activity also has high phenol and flavanoid content [15]. Anthocyanin are reported to have a significant antioxidant activities and inhibitory effect of lipid peroxidation [21]. The present study experiment the anthocyanin activity of *M. pruriens* of black and white seed varieties. Through these findings, the seeds of *M.pruriens* having rich content of anthocyanin.

The present experimental setup antioxidant activity of methanolic extract of the black and white seeds of *M.pruriens* and possible mechanism had been investigated by evaluating DPPH, superoxide radical scavenging activity, metal chelating activity also assessed. DPPH radicals are very popular and well established free radical used to investigate free radical scavenging power of components in vitro. The present study indicates strong DPPH radical scavenging activity of the extracts of black seeds varieties of *M.pruriens*. This finding was compared to some previous report, DPPH radical scavenging ability of methanolic extract of various plants *(A.calamus. H.antidysenterica* showed relatively poor antioxidant activity [18]. The scavenging activity of methanolic extract of *Halia bentony* also lower activity [2]. Superoxide anion radical has been known as relatively weak oxidant but ability to generate more toxic and dangerous single oxygen, hydroxyl radical and peroxynitrile radicals made superoxide radicals a dangerous reactive species [7]. The result of present study revealed a good significant superoxide radical scavenging activity of extract under evaluation. Ferrozine can quantitatively form complexs with Fe^{2+} but in the presence of ion chelating agents, the complex formulation is disrupted resulting in a reduction in the red color of the complex measurement of the rate of reduction of the color, therefore the allow estimation of chelating activity of the co-existing chelator. The data obtained from results that the extract of *M.pruriens* demonstrates an effective capacity for iron binding suggestion that the action as peroxidation protector may be related to its iron binding capacity. In this assay the extract and started compounds interfered with the formation of ferrous complex with reagent ferrozine suggesting that it has chelating activity of extract may be attributed due to the presence of endogenous chelating agents, mainly phenolics [10]. In the present study demonstrate, the antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen, flavanoids are wide spread in the natural compounds and posses a broad spectrum of biological activities.

The chemical composition phenol and flavanoids content of *M.pruriens* is higher which are known to possess antioxidant activities. The high phenolic and flavanoid content in the *M.pruriens* may be responsible for its free radical scavenging activity.

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