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Research Article

ANTIOXIDANT AND ANTICANCER ACTIVITY OF *MUCUNA COCHINCHINENSIS* (LOUR.) CHEVAL SEEDS

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ABSTRACT

Objective: *Mucuna cochinchinensis (Lour.)* Cheval (Leguminosae) commonly known as 'Lyon bean' is an annual twining herb with high content of L-dopa, a drug of choice in Parkinson's disease. Since the antioxidant property has significant role in many diseases, the present study was aimed to evaluate the *in-vitro* antioxidant and cytotoxic effect of *Mucuna cochinchinensis* seed extracts.

Methods: Standard *in-vitro* antioxidant assays such as reducing power, super oxide scavenging, nitric oxide scavenging and lipid peroxidation were performed. Anticancer activity was demonstrated using MTT assay on human cell lines.

Results: The *Mucuna cochinchinensis* seed extracts exhibit potent antioxidant effect compared to the standards on all the tested protocols. MTT assay revealed the potency of extracts as better anticancer agent.

Conclusion: To conclude, this study demonstrates the potentiality of *Mucuna cochinchinensis* seed in treatment of cancer.

Keywords: Anticancer, Antioxidant, *Mucuna cochinchinensis.*

INTRODUCTION

Oxidative damages are either removed or repaired by host antioxidant defense mechanisms [1] carried out by specific enzymes. Antioxidant properties are one of the most important claims for food ingredients, dietary supplements, cosmetics and anticancer natural products [2] since the therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytoconstituents. It has been clearly stated that, an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases were determined [3]. Because of multiple mechanism of actions and least toxic nature, plant based antioxidants are preferred rather than synthetic drugs [4,5]. Cancer do affect nearby cells and also very difficult in the treatment due to drug resistance, toxicity and low specificity [6]. Over 50% of the drugs in clinical trials for anticancer activity were isolated from natural sources or related to them [7] also been reported with antioxidant property. An widespread idea in screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases have been in current scenario.

Mucuna cochinchinensis (Family: Leguminosae., Synonum: Lyon bean) is an annual twining herb with white or pale purple flowers and glabrescent pods. It is widely distributed in the tropics and subtropics and cultivated mostly in Bengal and Bihar region of India for its edible pods and seeds. The fleshy and tender fruits of the plant are valued as vegetable [8]. The seed contains carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits of *M. cochinchinensis* yield L-dopa (0.96%), which is an important drug for Parkinson's disease [9]. *Mucuna pruriens* exhibit antioxidant property due to L-dopa related to its antiparkinsons activity is well understood [10]. The proximate composition and amino acid profile of *Mucuna cochinchinensis* suggested that it could be a promising nutritional supplement [11]. On view of this an attempt was made in this study to explore *Mucuna cochinchinensis* seeds for its antioxidant and anti cancer activity.

MATERIALS AND METHODS

Plant materials and Extraction

Mucuna cochinchinensis seeds were collected from Western Ghats, Tamilnadu, India and were identified and authenticated by renowned Botanist. A voucher specimen was kept in Department of Pharmacognosy, Ultra College of Pharmacy, Madurai (Voucher specimen No: UCP/11/031). The seeds were dried in shade and powdered in a mechanical grinder. About 250 g of dried seed powder was extracted by cold maceration using shaker for 72 hr in 1.0 L of methanol and 1.0 L of ethyl acetate [12]. The methanolic extract (MMC) and ethyl acetate extract (EMC) of *M. cochinchinensis* were concentrated in rotary vacuum evaporator and preserved in tightly closed container. The extracts thus obtained were directly used in the phytochemical screening [13] antioxidant and cytotoxic assays.

Total Phenolic and flavonoidal content

Folin's Ciocalteu method was adopted to estimate total phenolic content in the extracts [14] and total flavonoidal content was estimated using the method described by Chang., et al [15].

Antioxidant activity

Determination of reducing power

The reducing power was determined using the method described by Athukorala et al [16]. 1.0 ml extract is mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance is measured at 700 nm. Ascorbic acid [17] is used as a positive control.

Superoxide scavenging ability

The superoxide anion scavenging activity is measured as described by Robak and Gryglewski (1988) [18]. The superoxide anion radicals are generated in 3.0 ml of Tris- HCl buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). The reaction is started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and then the absorbance is measured at 560 nm against a blank sample and ascorbic acid [19] is used as a positive control.

Nitric oxide method

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent. 3.0 ml of 10 mM

sodium nitroprusside in phosphate buffer is added to 2.0 ml of extract and reference compound in different concentrations (20 - 100 µg/ml). The resulting solutions are then incubated at 25°C for 60 min. A similar procedure is repeated with methanol as blank, which serves as control. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) is added and absorbance of the chromophore formed is measured at 540 nm. Percent inhibition of the nitric oxide generated is measured by comparing the absorbance values of control and test preparations. Ascorbic acid, used as a positive control [20].

Lipid peroxidation

Egg yolk was separated out and washed with acetone until yellow color disappears. The creamy white powder thus obtained is used for the procedure by dissolving in phosphate buffer pH 7.4 (3mg/ml). The reaction mixture containing egg lecithin (1ml), ferric chloride (0.02ml), ascorbic acid (0.02ml) and extract or standard (0.1ml) in DMSO at various concentrations was kept for incubation for 1 hour at 37ºC. After incubation 2 ml of 15% TCA and 2ml of 0.37% TBA were added. Then the reaction mixture was boiled for 15 min, cooled, centrifuged and absorbance of the supernatant was measured at 532 nm. [21].

Determination of anticancer activity- MTT assay

The experiment was performed as described by Francis & Rita [22]. Briefly, the cancer cells were seeded in 96-well plates at a density of 1×10^4 cells/well in 100 µl RPMI. After twenty-four hours of seeding, the medium was removed and then the cells were incubated for 3 days with RPMI with the absence and/or the presence of various concentration of *M.cochinchinensis* extracts. Extracts were added at various concentrations ranging from 62.5-1000 µg/ml. After incubation, 20 µl of MTT reagent was added into each well. These plates were incubated again for 4 h in CO_2 incubator at 37°C. The resulting MTT-products were determined by measuring the absorbance at 570 nm using ELISA reader. The cell viability was determined using the formula: Viability % = (optical density of sample/optical density of control) × 100. IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

RESULTS AND DISCUSSION

In this study *M.cochinchinensis* seeds were evaluated for its potency as an antioxidant and anticancer activity. Two different extracts *viz*. methanolic (MMC) and ethyl acetate (EMC) were selected for this study, as both extracts in many studies reported to have solublizing ability of most of the secondary metabolites. The preliminary phytochemical screening of MMC and EMC revealed the presence of alkaloids, phenols, flavonoids, amino acids, quinones, steroids and carbohydrate. MMC and EMC showed high concentrations of phenolic and flavonoid content (Table 1).

The total flavonoidal content is rich in EMC (16.884±0.157 mE Quercetin/mg dry extract) compared to MMC (4.093±0.031 mE Quercetin/mg dry extract). Recent evidence has shown that flavonoids have evolved particular roles in legumes [23]. The bioactivity of flavonoids and related polyphenols appears to be mediated through a variety of mechanisms, though particular attention has been focused on their direct and indirect antioxidant actions. Antioxidants may protect cell constituents against oxidative damage and thereby limit the risk of various degenerative diseases associated to oxidative stress [24].

Table 1: Total phenolic and flavonoid content of MMC and EMC

Total phenolic content (mE GAE/mg dry extract)		**Total flavonoid content (mE Quercetin/mg dry extract)**	
MMC	EMC	MMC	EMC
4.96±0.048	6.56±0.051	4.093±0.031	16.884±0.157
Slope: Y=0.025X+0.0580		Slope: Y=0.0391X+0.040	
Regression: R^2= 0.9952		Regression: R^2= 0.9830	

MMC, EMC- Methanolic and Ethyl acetate extracts of *Mucuna cochinchinensis* seeds, GAE- Galic acid

Table 2: Antioxidant effects of MMC and EMC

Methods	**MMC**		**EMC**		**Standard**	
	IC_{50} (µg/ml)	**R^2**	**IC_{50} (µg/ml)**	**R^2**	**IC_{50} (µg/ml)**	**R^2**
Reducing power	88.5± 0.60	0.936	85.67±0.59	0.976	43.8±0.30	0.958
Super oxide	46.53± 0.50	0.997	56.17±0.65	0.987	31.6±0.60	0.944
Nitric oxide	46.0±0.50	0.995	56.73±1.53	0.981	31.77±0.75	0.928
Lipid peroxidation	586.96±0.27	0.978	29.63±0.57	0.962	232.33±2.52	0.929

MMC, EMC- Methanolic and Ethyl acetate extracts of *Mucuna cochinchinensis* seeds

IC_{50}- represents the 50 % inhibitory concentration, R^2 – represents the regression.

From Table 2 it is evident that MMC and EMC exhibit good antioxidant property under various mechanisms tested. MMC showed high superoxide and nitric oxide scavenging ability compared to EMC. Due to high reactivity of the hydroxyl group in flavonoids, radicals are made inactive [25]. Interestingly, nitric oxide can be viewed as a radical itself, and it is reported that nitric oxide molecules are directly scavenged by flavonoids [26]. When flavonoids are used as antioxidants, free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage [27]. EMC proved to exhibit potent lipid peroxidation (29.63±0.57 µg/ml) even when compared to standard (232.33±2.52 µg/ml). Alcoholic extract of *Mucuna pruriens* is reported to have lipid peroxidation ability in both stress induced and alloxon induced *in-vivo* models [28]. Lipid peroxidation of alcoholic extract of *M.pruriens* seed increased in increasing concentrations [29]. All the reactive oxygen species can damage DNA, and division of cells with unrepaired or misrepaired damage leads to mutations. If these changes appear in critical genes, such as oncogenes or tumor suppressor genes, initiation or progression may result. Reactive oxygen species can interfere directly with cell signaling and growth. The cellular damage caused by reactive oxygen species can induce mitosis, increasing the risk that damaged DNA will lead to mutations, and can increase the exposure of DNA to mutagens [30]. So antioxidant is very much associated with anticancer activity. Enhanced antioxidant mechanism and anticancer effect were reported for a flavone lupeol [31]. Methanolic extract of *M.pruriens* exhibit significant antitumor and antioxidant effect in Erlich Ascites Carcinoma (EAC) bearing mice [32]. In this study MMC and EMC were tested for their anticancer potency against human cancer cell lines Hela, Hep 2, MCF 7 and NIH 3T3 Mouse embryonic fibroblasts cells using MTT assay.

Table 3: Cytotoxic effect of MMC and EMC in human cell lines

Parameters	MMC				EMC			
	Hela	Hep2	MCF 7	NIH 3T3	Hela	Hep2	MCF 7	NIH 3T3
IC_{50} µg/ml	>1000	>1000	682.0 ± 2.0	>1000	257.19 ± 0.27	218.7 ± 0.62	221.06 ± 0.16	>1000
R^2	0.962	0.982	0.923	0.938	0.992	0.982	0.982	0.952

MMC, EMC- Methanolic and Ethyl acetate extracts of *Mucuna cochinchinensis* seeds

Hela- Human cervical cancer cell line, Hep2- Human laryngeal epithelial carcinoma cells , MCF7- Breast cancer, NTH 3T3- Mouse embryonic fibroblasts (Standard)

IC_{50}- represents the 50 % inhibitory concentration, R^2 – represents the regression.

The results (Table 3) revealed that EMC showed potent IC_{50} value for all tested cell line except NIH 3T3 normal cells (IC_{50} > 1000 µg/ml) whereas MMC doesn't produce any significant effect compared to EMC. Thereby this study suggests that the antioxidant and anticancer property of *Mucuna cochinchinensis* seed extracts may be due to its rich flavonoidal content.

CONCLUSION

From the results obtained we are able to conclude that ethyl acetate extract of *Mucuna cochinchinensis* seed had significant antioxidant and anticancer activity. The components responsible for the activity are unclear. Further studies are required to isolate the exact constituents and to understand their mechanism**.**

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