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Research Article

ANTIOXIDANT STATUS ANTICANCER AND HEPATO PROTECTIVE EFFICACY OF *MACROTYLOMA UNIFLORUM* SEEDS AN IN-VITRO APPROACH

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ABSTRACT

The *Macrotyloma uniflorum* seed (Horse gram) has been underutilized pulse around the world. Nevertheless, it contains most valuable nutritional and pharmacological phytochemicals. Thus, this research was performed to evaluate the antioxidant, anticancer, and hepatoprotective potential of horse gram methanol extract. Interestingly, methanol extract of horse gram demonstrated dose dependent antioxidant, anticancer, and hepatoprotective activities. Since, at increased (100 mg mL^{-1}) concentration the methanolic extract scavenged the DPPH radical up to 93.5% and reduced the ferric (Fe^{3+}) into ferrous (Fe^{2+}) up to 95%. Similarly, the methanol extract also possess considerable anticancer activities up to 75.64% at 300 mg mL^{-1} concentration. Furthermore, at increased concentration (300 mg mL^{-1}), the methanol extract effectively protect (hepatoprotective) the HEK293 cells (95.71%), which exposed to 100 mM glucose (hepatotoxicity). The obtained results conclude that the horse gram methanol extract contains pharmacologically valuable phytochemicals and that regular intake of horse gram can be recommended to improve human health.

Keywords: *Macrotyloma uniflorum*, Antioxidant, Anticancer, Cytotoxicity, Nephroprotectivity.

INTRODUCTION

Regular consumption of phytochemical-enriched cereals, grains, vegetables, and fruits can help to reduce the harm caused by free radicals and that are responsible for a variety of chronic illnesses including cancer, vascular disease, coronary heart disease, macular degeneration, metabolic syndrome, rheumatism, immunodeficiency syndrome, ageing, and so on (Golzarand *et al*., 2015). The ability of antioxidant components such as flavonoids, tannins, and phenolic acids to serve as efficient antioxidants has received a lot of attention recently (Gulcin, 2020). Grains of crops play a significant role in several regions of the world's as largest traditional diets since they are low in calories and high in protein, soluble fibres, a wide range of phytonutrients, and phyto-constituents (Chivenge *et al*., 2015). The extracts derived from grains contains nutritional tannins as well as non-tannin polyphenolic compounds is prominent among villagers in certain regions of India (Taylor and Duodu, 2017). Consumption of these grains have been associated with a lower threat of diabetes, obesity, as well as an inhibiting role in the prevention of cardiovascular disease (Asgary *et al*., 2018). A nutritional supplement would be any compound, which is a nutrition or an element of such a food that contains health - related benefits, such as disorder prevention and cure (Giampieri *et al*., 2014). Nutraceuticals include isolated nutrition, dietary supplements, particular diet plans, herbal supplements, processed foods, and processed refreshments (Mondello, 2013). Polyphenolic components of numerous grains have been investigated by several researchers as well as found to already have prospective medicinal benefits, such as antioxidant activity (Saleh *et al*., 2013). Thus, the research into the significance of non-nutrient components such as phenols, tannins, and flavonoids found in plant products, as natural antioxidants has increased significantly (Hano and Tungmunnithum, 2020). The *Macrotyloma uniflorum* is widely grown, particularly in waterless areas of various countries

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including India (Fuller and Murphy, 2018). Previous research has shown that *M. uniflorum* seed is a fine source of amino acids, protein, carbohydrates, low lipid, iron and molybdenum (Bhaskar *et al*., 2019). It also contains slow metabolizable carbohydrate (starch), which has a reduced postprandial blood glucose reaction while ingested by diabetics (Chahota *et al*., 2020). Furthermore, a solvent extract of horse gram plants has been reported as hypolipidemic activity in lab animals fed a elevated fat diet (Kumar *et al*., 2013). The extract administered to rabbits with oxidative stress caused by an elevated fat diet resulted in an increase in superoxide dismutase (SOD) as well as catalase (CAT), as well as an increase in lowered glutathione (GSH) accumulation (Jarukamjorn *et al*., 2016). Such horse gram has been consumed by a massive population in rural areas of southern India as a whole seed sprouts (Chahota *et al*., 2020). Notwithstanding, consuming horse gram seeds upon having to process including heating accompanied by preparing meals, together with boiled rice, millet, and sorghum is indeed a most common practise in rural Indians (Platel and Srinivasan, 2016). Furthermore, the food preparation alcohol of horse gram seedlings with ingredients is thought to become a prospective cure for the throat infection, fever, common cold, as well as the soup produced from this plant's seedlings was shown to produce heat and assist solubilize renal stones (Tamang *et al*., 2020). However, as per the author's knowledge, the cytotoxicity, and hepatoprotective efficiency of methanolic extract of horse gram against HepG2 and HEK 293 cells respectively have not been reported. Thus, to understand the possible health benefits of regular consumption of horse gram is critical to enhance the health of common peoples. Accordingly, this study was framed to evaluate the free radicals scavenging (DPPH, ABTS, OH, and FRAP), cytotoxic (HepG2 cells) and hepatoprotective (HEK 293 cells) activity of methanol extract of horse gram through in-vitro approach.

MATERIALS AND METHODS

Extract preparation

The *M. uniflorum* seed was collected from nearby market and pulverized to obtain fine powdered particles for extract preparation. The standard hot plate extraction method was followed for extraction process. Concisely, about 100 g of well pulverized and sieved *M. uniflorum* seed sample was completely dissolved in 100 mL of methanol solvent in a 250 mL conical flask and kept for overnight extraction at 50 ± 1°C. The methanol solvent from extract was then allowed to evaporate until completely dried. Well dried sample was dissolved in dimethyl sulfoxide (DMSO) to obtain various dosages (12.5, 25, 50, 75, and 100 mg mL^{-1}) for further studies.

Antioxidant activity potential analysis

DPPH assay

The free radical scavenging efficiency of 12.5 - 100 mg mL^{-1} concentrations of methanolic extract was investigated through the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity by adopting the protocol of Aksoy et al. with slight modifications (Aksoy *et al*., 2013). Briefly, about 100 µL of (each) DPPH reagent and various concentrations of test samples were dissolved and left undisturbed for 30-35 min at ambient temperature. Similarly, 100 mg mL^{-1} concentrations of ascorbic acid (vitamin C) were utilized as a positive control. Then absorbance was measured with 517 nm using a UV-vis. spectrophotometer. The typical equation was applied to determine the percentage of DPPH scavenging and IC_{50} value was calculated through linear regression study.

Hydroxyl (OH) radical scavenging activity

The hydroxyl radical scavenging potential of various concentrations (12.5 - 100 mg mL^{-1}) of test samples were studied by adopting the modified protocol of Pavithra and Vadivukkarasi (Pavithra and Vadivukkarasi, 2015). Concisely, 0.2 mL (each) of various concentrations of samples was blend individually with following ingredients: phosphate buffer: 0.8, $FeCl_3$: 0.2, EDTA: 0.2, 2-deoxy-d-ribose: 0.2 mL and mixed thoroughly. Such reaction mixtures were then kept over the water bath for few min and then added vitamin C and H_2O_2 (each 0.2 mL). After 60 min of incubation at 37 °C, ice cold thiobarbituric acid (1.5 mL) and 25% HCl (1.5 mL) were mixed and then heated at 100 °C for 15 min. Subsequently (after cooled), absorbance of these reaction mixtures (test and 100 mg mL^{-1} concentration of Catechin as positive control) were recorded at 532 nm. The hydroxyl radical scavenging % and IC_{50} values were standard formula and linear regression studies respectively.

ABTS assay

The ABTS free radical scavenging potential of different concentrations (12.5 - 100 mg mL^{-1}) of methanolic extract was investigated by adopting the protocol of Proestos et al. with slight modifications (Proestos et al., 2013). In brief, about 100 µL of each dosage of sample was added with 100 µL of ABTS reagent individually and kept for 20 min at normal temperature. The 100 mg mL^{-1} concentration of vitamin C was utilized as reference. The absorbance of each reaction mixtures were then read 734 nm and used the standard formula and linear regression to calculate the ABTS radical scavenging percentage and IC_{50} values of test and positive control.

Ferrous reducing antioxidant power activity (FRAP)

The ferrous reducing competence of various dosage of test sample was analyzed by adopting the procedure of Rahman et al. with slight modifications (Rahman et al., 2015). Concisely, 0.25 mL (each) of various concentrations (12.5 - 100 mg mL^{-1}) of sample was added with 2.5 mL (each) of 1% $K_4[Fe(CN)_6]\cdot 3H_2O$ and 2.5 mL of potassium buffer. Such mixtures were left undisturbed for 20 min at **50 ± 1**°C, later 0.5 mL of TCA (10%) was added individually to each reaction mix to stop further reduction and then centrifuged at 5000 rpm for 15 min. Subsequently, 1.8 mL

of test sample (supernatant) was blended with distilled water (0.35 mL) and $FeCl_3$ (0.1%) reagent. The 100 mg mL^{-1} dosage of quercetin was utilized as reference. The absorbance of these reaction mixtures were recorded at 700 nm. The standard formula and linear regression analyses were used to calculate the ferric reducing percentage and IC_{50} values of test sample and positive control.

Anticancer activity potential analysis

The anticancer activity potential of methanol extract test sample was studied by MTT assay based cytotoxicity study on Liver Hepatocellular Carcinoma (HepG-2) cell line by following the methodology of Al-Sheddi et al. with slight modification on Minimal Essential Medium (MEM, GIBCO) (Al-Sheddi et al., 2018). Briefly, about 5000 viable HepG-2 cells were seeded on 96 well plate (Triplicates) and then incubated for overnight at 37 ±1 ºC. After incubation, the attached cells were gently washed with 100 mL of medium (serum-free) and then left starved for 1 h at 37 ºC. Subsequently, 100 mL of various concentrations (12.5 - 300 mg mL^{-1}) of test sample were treated with HepG-2 cells and then incubated for overnight at 37 ºC. Then the medium was aspirated then and refilled with MTT reagent and again incubated for 4 h at 37 ºC in a CO_2 chamber. After incubation, the medium was removed and attached cells were gently rinsed with PBS. Then the cells were suspended in 100 mL of DMSO and then stained with formazan and mixed well. Later, the cell-loaded 96 well plates were recorded at 570 nm. The anticancer drug Melphalan was used as a reference control. The following equation was used to calculate the percentage of cell viability.

$$\text{Percentage of cell viability} = \frac{\text{Absorbance of sample (Methanol extract)}}{\text{Absorbance of sample (Un treated)}} \times 100$$

Hepatoprotective potential analysis

Hepatoprotective activity potential of test sample was studied by following the methodology of Amalia *et al*., with slight modification (Amalia *et al*., 2021). Briefly, about 50 mL of diluted (5000 cells/well) HEK 293 cells was added in 96 well microplate containing DMEM medium and incubated for overnight at 37 ºC in a CO_2 (5%) chamber. After overnight incubation, the (supernatant discarded) monolayer cells was rinsed with DMEM. Then, 50 mL of various concentrations (12.5 - 300 mg mL^{-1}) of methanolic extract were individually added and then incubated for 1 h in a CO_2 (5%) incubator at 37 ºC, and then added 50 mL of 100 mM glucose (as nephrotoxic inducer) solution and incubated for 24 h. Subsequently, 10 mL of water-soluble tetrazolium-8 solution was added and incubated at 37 ºC in a CO_2 (5%) incubator for 2 h. Then 100 mL of HCl was blended to each well to inhibit the reaction and then the absorbance of was read at 450 nm.

RESULTS AND DISCUSSION

The antioxidant efficiency of methanolic extract sample was investigated against number of free radical, which including DPPH, OH, ABTS, and FRAP through in-vitro approach (Table 1). The horse gram methanol extract possess considerable DPPH free radicals scavenging potential on dose dependent basis. Since, at increased concentration of 100 mg mL^{-1} the DPPH free radicals was scavenged up to 93.5%, interestingly it was close to the DPPH radicals scavenging potential (98.95%) of ascorbic acid (positive control) (Figure 1). Moreover, the IC_{50} was calculated as 41.524 mg mL^{-1} and it almost close to the IC_{50} of positive control (38.74 mg mL^{-1}). Such result suggests that the horse gram methanol extract may contain significant quantity of bioactive compounds with antioxidant potential. Interestingly, a report stated that the acetone (70%) extract and followed by methanol extract possess excellent free radicals scavenging on DPPH, and ABTS free radicals as well as fine ferric reducing potential at increased concentration (Siddhuraju and Manian, 2007). The bioactive components present in the horse gram methanol extract can act as a H atoms donor and it can interact with DPPH (unstable) radical and convert it as DPPH-H (stable) substance and it can also reduce the toxic effects (Moukette *et al*., 2015). The elevated quantity of polyphenolic group bioactive components might enhance the antioxidant activity (Karaś *et al*., 2017). Since, numbers of reports suggests that the antioxidant efficiency of plant is determined by the occurrence of polyphenols (Hajimahmoodi *et al*., 2010; Zhang *et al*., 2018). Such polyphenols can prevent the formation and accumulation of unstable free radicals and that are accountable for the cause of number of chronic diseases and oxidative stress (Losada-Barreiro and Bravo-Diaz, 2017). People who consume a polyphenolic-rich nutrition have a lower risk of heart disease and cancer (Cruz *et al*., 2016).

Table 1. Absorbance of methanol extract on various free radicals (DPPH, OH, ABTS, and FRAP,) scavenging activity.

Plant extract	Various concentration of methanol extract (mg mL^{-1})					
	12.5	25	50	75	100	Positive control (100 mg mL^{-1})
DPPH	2.47 ± 0.06	2.95 ± 0.03	3.41 ± 0.07	3.69 ± 0.05	3.95 ± 0.10	4.23 ± 0.09 (Ascorbic acid)
OH	0.95 ± 0.09	1.24 ± 0.05	1.58 ± 0.02	1.74 ± 0.07	1.98 ± 0.04	4.18 ± 0.08 (Catechin)
ABTS	0.85 ± 0.05	1.02 ± 0.02	1.28 ± 0.04	1.52 ± 0.06	1.94 ± 0.07	4.04 ± 0.03 (Ascorbic acid)
FRAP	1.94 ± 0.02	2.01 ± 0.05	2.61 ± 0.03	2.89 ± 0.02	3.11 ± 0.04	4.01± 0.04 (Quercetin)

The values mentioned in the table are mean and standard error (±SE) of triplicates

A most reactive oxygen-centered species is the OH (hydroxyl) radical, which causes significant damage to nearby biomolecules (Raedschelders *et al*., 2012). An increased concentration (100 mg mL^{-1}) of methanolic extract demonstrated moderate OH radicals scavenging potential as 58.89% and interestingly it was comparatively lower than the antioxidant activity (98.95%) of Catechin (Figure 2). Furthermore, the IC_{50} value was calculated and recognized as poor (76.55 mg mL^{-1}) than positive control (35.61 mg mL^{-1}). Hydroxyl (OH) radicals are established by developing a Fe+3-EDTA premixture along with vitamin c as well as H_2O_2 at pH 7.4, resulting in the degradation of 2-deoxy-d-ribose and the formation of a malondialdehyde like product (Manjari *et al*., 2018). This hydroxyl radical can be scavenged by sequential electron and proton transmission from the extract's oxidant molecules (Bayat and Fattahi, 2018). According to this, the methanolic extract of *M. uniflorum* seed may contain lowest quantity of electron and proton donating oxidant molecules (Naji and Devaraj, 2011), hence moderate hydroxyl radicals scavenging activity was observed. Accordingly the phytochemicals such as anthocyanins, kaempfetol, isoflavones, quercetin, myricetin, and flavonol enriched plant extract showed remarkable hydroxyl radicals scavenging activity (Sreerama *et al*., 2010). The result obtained from this study revealed that the methanolic extract of *M. uniflorum* seed possess moderate ABTS radicals scavenging activity even at 100 mg mL^{-1} concentration. Since, at this elevated concentration, the methanolic extract scavenged the ABTS radical up to 52.65% and it was comparatively lower than the antioxidant activity (95.21%) of ascorbic acid. The IC_{50} value for ABTS scavenging activity of methanolic extract was calculated as 80.08 mg mL^{-1} and it found that as least than the IC_{50} value of positive control (34.27 mg mL^{-1}). This result suggests that, the methanolic extract may have lowest number of electron donating active components that react with ABTS radicals. Since, this ABTS assay is electron (from bioactive compounds) transfer based assay. It was confirmed by shifting the electrons from bioactive compounds and interact with the dark blue color $ABTS^{+}$ compound and reduced into colorless ABTS substance (Macchioni *et al*., 2021). Interestingly, Parikh and Patel reported that the flavonoid, and phenolic enriched various pulses as well as split pulses frequently consumed by people around the world possess remarkable health condition (Parikh and Patel, 2018). Since, these phenol and flavonoid enriched diet can reduce the free radicals formation and accumulation in the cells, which resulting improved health condition in humans (Parikh and Patel, 2018; Wojtunik-Kulesza *et al*., 2016).

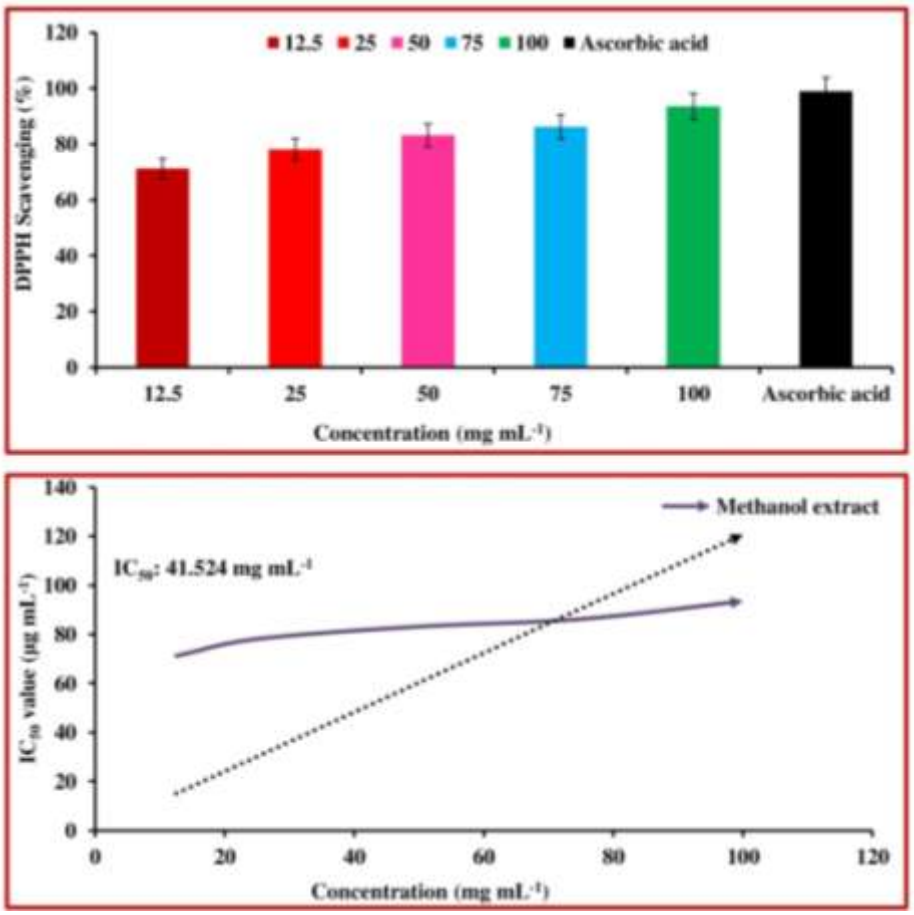

Figure 1. DPPH radicals scavenging potential of methanol extract of horse gram. The values mentioned in the figures are mean and standard error (±SE) of triplicates.

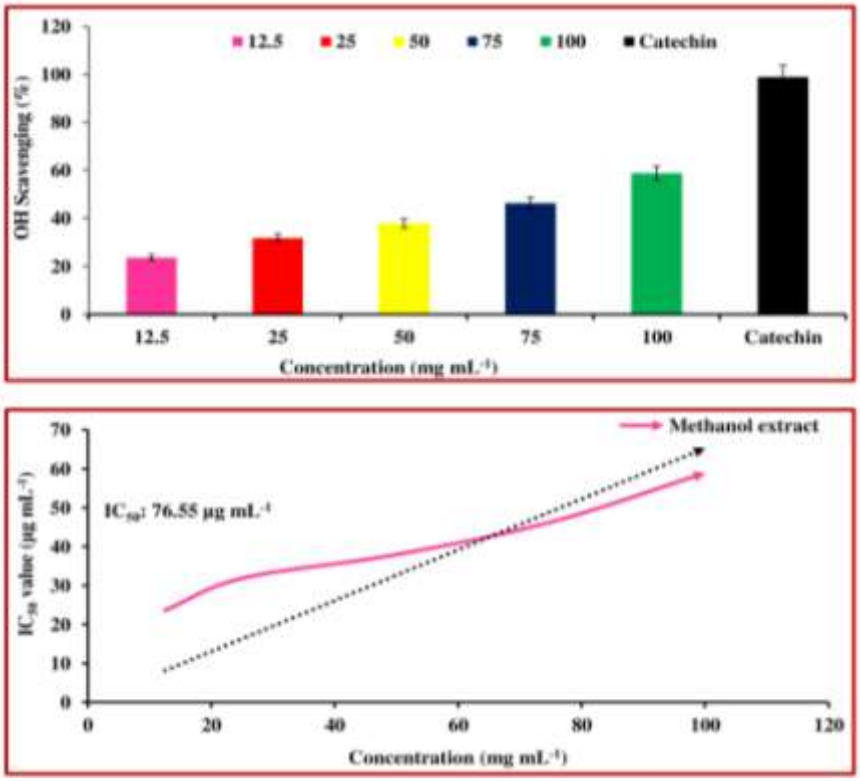

Figure 2. Hydroxyl (OH) radicals scavenging competence of methanol extract of horse gram. The values mentioned in the figures are mean and standard error (±SE) of triplicates.

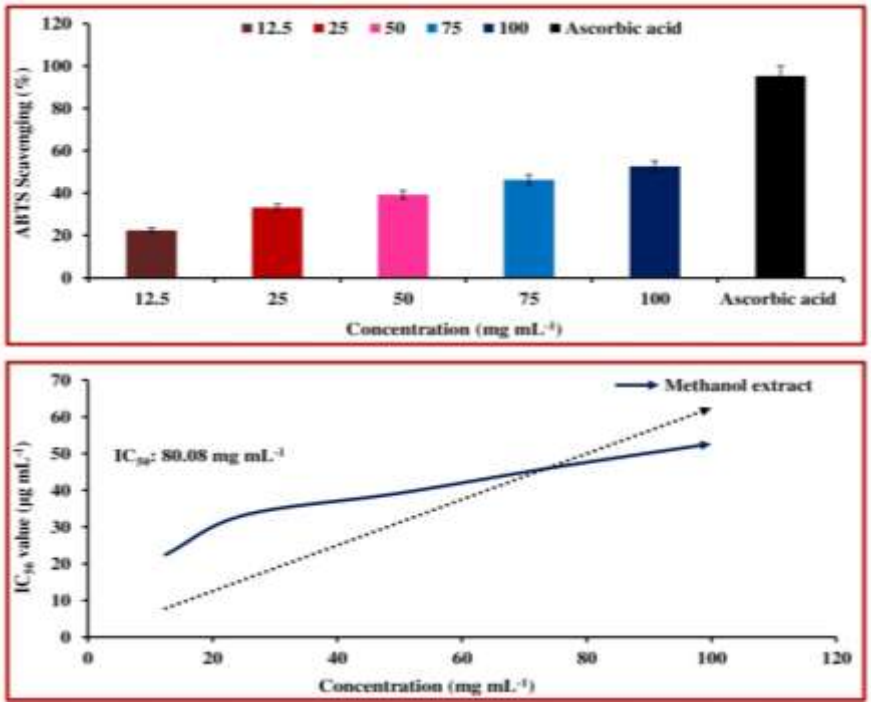

Figure 3. ABTS radicals scavenging potential of methanol extract of horse gram. The values mentioned in the figures are mean and standard error (±SE) of triplicates.

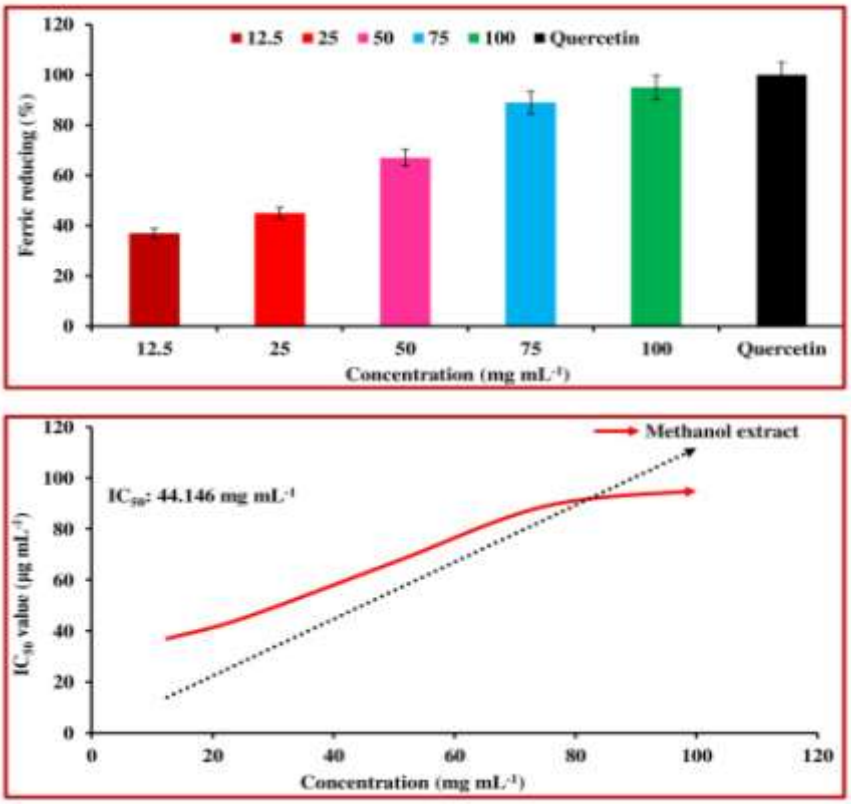

Figure 4. Ferric reducing potential of methanol extract of horse gram. The values mentioned in the figures are mean and standard error (±SE) of triplicates.

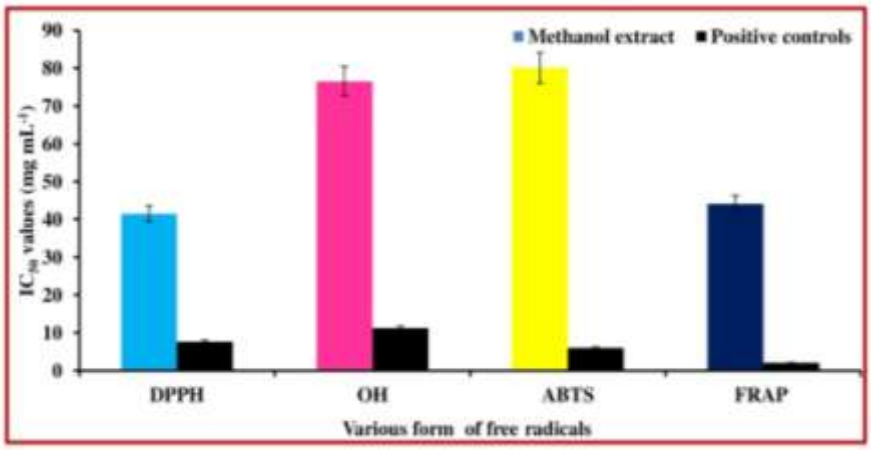

Figure 5. IC_{50} values of methanol extract against various free radicals: DPPH, OH, ABTS, and FRAP. The values mentioned in the figures are mean and standard error (±SE) of triplicates.

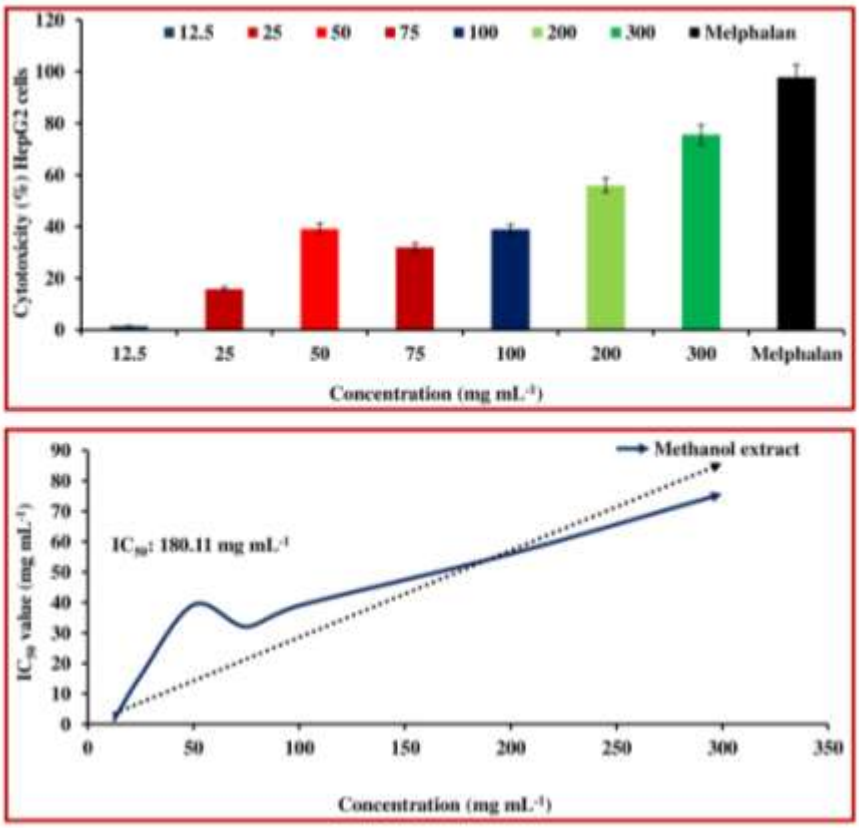

Figure 6. Anticancer activity of methanol extract against HepG2 cells: Through cytotoxicity study. The values mentioned in the figures are mean and standard error (±SE) of triplicates.

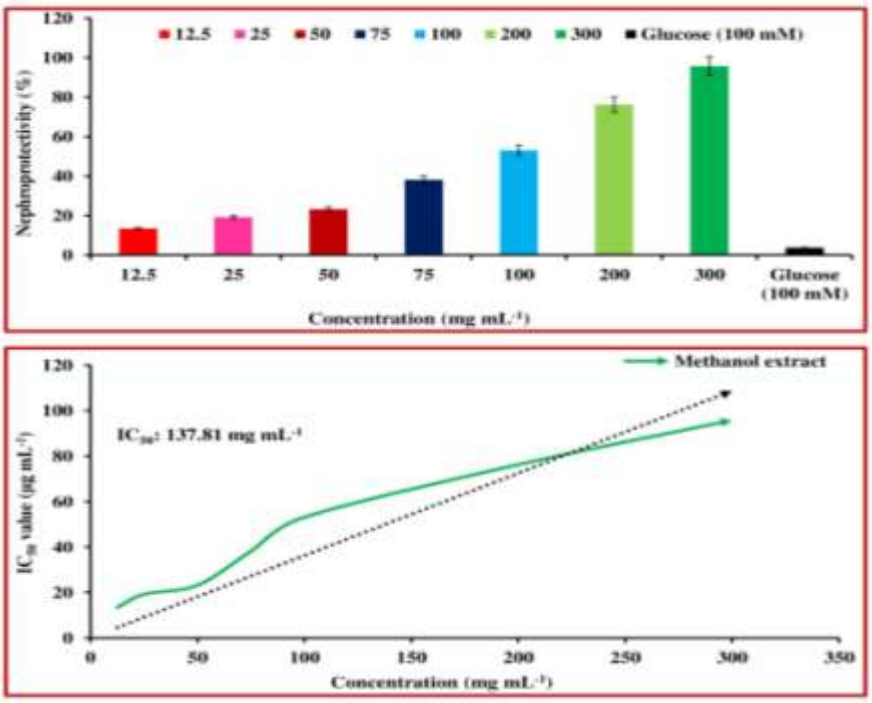

Figure 7. Hepatoprotective activity of methanol extract on glucose induced nephrotoxicity on HEK 293 cells. The values mentioned in the figures are mean and standard error (±SE) of triplicates.

Table 2. Anticancer activity (Cytotoxicity) profile of methanol extract on HepG2 cells studied through MTT assay.

Sample	Treated Conc. mg mL^{-1}	Absorbance at 570nm (ELISA Reader)	Percentage of HepG2 cell viability	Number of viable HepG2 cell counted	Percentage of cytotoxicity
Methanol extract plant sample	12.5	1.2564 ± 0.1	98.48 ±1.0	4924 ± 4	1.52 ± 0.56
	25	0.9852 ± 0.1	84.16±2.6	4208 ± 6	15.84 ± 1.1
	50	0.5864 ± 0.2	68.02 ±2.9	3401 ± 5	31.98 ±2.5
	100	0.5216 ± 0.4	61.08±1.5	3054 ± 8	38.92 ± 1.7
	200	0.4758 ± 0.3	44.08 ± 1.5	2204 ± 6	55.92 ± 1.3
	300	0.4214 ± 0.6	24.36 ±1.8	1218 ± 8	75.64 ± 1.5
Melphalan (control)	35:5:60 v/v	0.105 ± 0.2	2.14 ± 0.6	107 ± 2	97.86 ± 0.4

The values mentioned in the table are mean and standard error (±SE) of triplicates

Table 3. Hepatoprotectivity profile of various concentration of methanol extract against glucose induced nephrotoxicity on HEK 293 cells.

Sample	Treated Conc. mg mL^{-1}	Absorbance at 570 nm (ELISA Reader)	Percentage of HEK 293 cell viability/ Hepatoprotectivity	Number of viable HEK 293 cell counted
Methanol extract plant sample	12.5	0.3782 ± 0.7	13.42 ± 0.98	783 ± 9
	25	0.4136 ± 0.4	19.16 ± 1.7	958 ± 7
	50	0.4628 ± 0.8	23.16 ± 1.6	1158 ± 5
	75	0.5114 ± 0.6	38.11 ± 2.4	1912 ± 8
	100	0.5753 ± 0.5	53.08 ± 2.0	2654 ± 5
	200	0.9624 ± 0.3	76.22 ± 1.4	3811 ± 4
	300	1.1652 ± 0.7	95.71 ± 2.1	4788 ± 3
Glucose (Hepatotoxic agent)	100 mM	0.135 ± 0.5	3.7 ± 1.3	185 ± 2

The values mentioned in the table are mean and standard error (±SE) of triplicates

dosage of 100 mg mL^{-1}, the sample effectively reduced the ferric molecule up to 95.52% and it was almost close to the ferric reducing potential (99.12%) of quercetin (Figure 4). A nominal IC_{50} value (44.146 mg mL^{-1}) was found for methanol extract to reduce the ferric into ferrous and it was considerably comparable with the IC_{50} value (42.371 mg mL^{-1}) of quercetin. The figure 5 represent the IC_{50} values of sample against various form of free radicals, which including DPPH, OH, ABTS, and FRAP. The obtained result suggests that, the methanol extract of *M. uniflorum* seed possess sufficient volume of ferric reducing antioxidants (Rajagopal *et al*., 2017). Since, antioxidant molecule converts (through electron transfer reaction) the ferric (Fe^{3+}) into ferrous (Fe^{2+}) (Zhou *et al*., 2021). While this ferric (Fe^{3+}) salt is reduced to ferrous (Fe^{2+}) salt, it produces a blue colour, the intensity of this can be measured using spectrophotometer (Bhatta *et al*., 2017). The oxidation state of ferric (Fe^{3+}) salt is similar to that of the radical cation $ABTS^{+}$ (Li *et al*., 2012). The anticancer activity potential of test sample was investigated through cytotoxicity study by MTT assay. Interestingly, the horse gram methanol extract demonstrated a dose dependent cytotoxicity on HepG2 cells. Such cytotoxicity activity against the HepG2 cells was ranged from 1.52 to 75.64% at a dosage of 12.5 – 300 mg mL^{-1} (Figure 6). Positive control melphalan showed cytotoxicity up to 97.86% against this HepG2 cells at 300 mg mL^{-1} concentration (Table 2). The IC_{50} value of methanol extract of horse gram against the HepG2 cells was found as 180.11 mg mL^{-1} and this value was moderately comparable with the IC_{50} value (137.23 mg mL^{-1}) of melphalan. A report stated that the butyrate enriched pulses can act as a protective shield against colorectal cancer (Prasad and Singh, 2015). Since, such bioactive components can minimize the menace of malignant related alteration in the cells (Seidel *et al*., 2017). Horse gram containing soluble and non-soluble bioactive components can aid the proper metabolic and other activities of cells, thus it may obstruct malignant cell formation (Gupta *et al*., 2019). The bioactive components belongs to the flavonoid group categories have been well recognized as possess considerable free radicals scavenging activity, anticarcinogenic, anti - allergic, as well as gastroprotective characteristics (Xuan and Khanh, 2016).

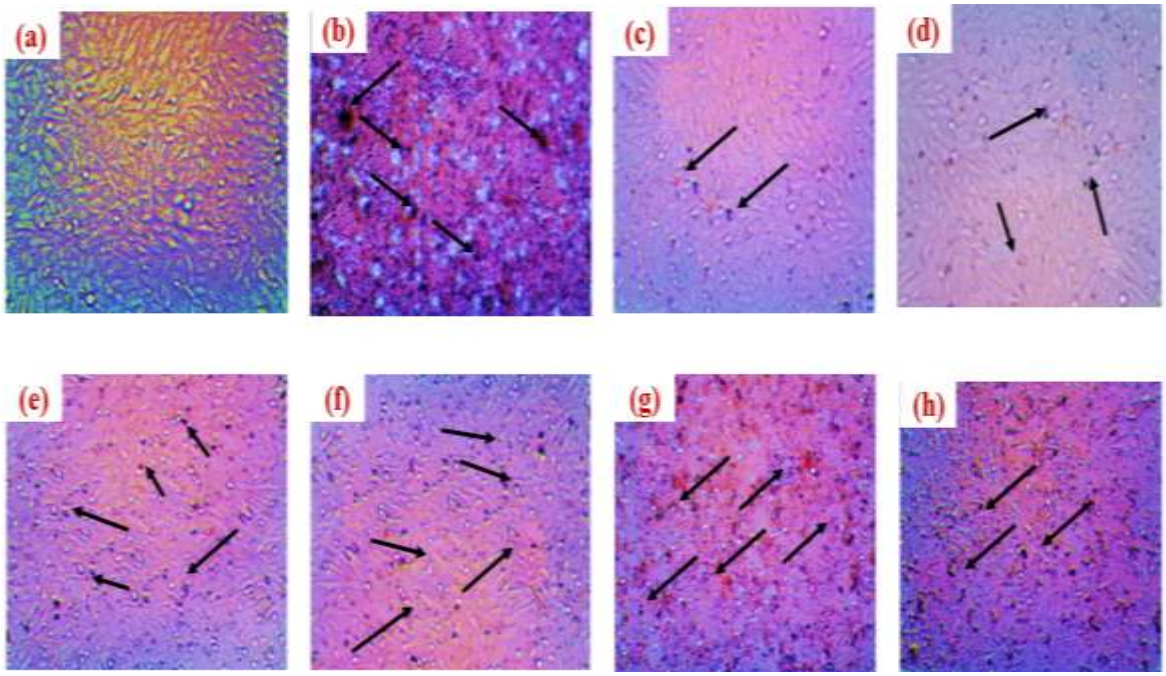

Figure 1. Cytotoxicity profile of methanol extract on HepG2 cell line.

Cytotoxicity (anticancer) potential of various concentrations of methanol extract of test plant sample against human liver cancer cell line HepG2. (a) Non treated (b) anticancer drug (melphalan) treated (c) 12.5 µg mL^{-1} treated (d) 25 µg mL^{-1} treated; (e) 50 µg mL^{-1} treated; (f) 100 µg mL^{-1} treated (g) 200 µg mL^{-1} treated; (h) 300 µg mL^{-1} treated. Arrow marks indicates cell lysis.

Interestingly, the methanolic extract demonstrated fine ferric reducing potential as on dose dependent basis. Because, at a The horse gram methanol extract effectively protect the HEK 293 cells from 100 mM glucose induced nephrotoxicity. The concentration of methanol extract determined the hepatoprotective percentage. The hepatoprotective activity percentage was ranged from 13.42% to 95.71% at the dosage of 12.5 – 300 mg mL^{-1} (Table 3). Interestingly, the obtained result suggest that, at elevated dosage (300 mg mL^{-1}) the test sample showed remarkable hepatoprotective efficiency (95.71%) on glucose (nephrotoxic) induced HEK293 cells (Figure 7). Interestingly, the Patel and Acharya reported that the horse gram possess significant biomedical potential to treat the urolithiasis and other kidney related urinary diseases (Patel and Acharya, 2020). In general, the crop seeds are enriched with number of pharmaceutically valuable phytochemicals than other plant parts. Such phytochemicals derived bioactive compounds have been reported as possess fine nephroprotective and hepatoprotective activities (Prasad and Singh, 2015). This suggests that the methanolic extract of *M. uniflorum* seed might possess sufficient quantity of hepatoprotective bioactive compounds. Accordingly, Sharma et al. reported about the number of nutraceutical properties of horse gram (Sharma *et al*., 2019). As per the author's knowledge, this is the first report about hepatoprotective activity of horse gram on glucose induced nephrotoxicity in HEK293 cells. Another report stated that the *Dolichous biflorus* seeds possess considerable hepatoprotective activity on Ethylene Glycol Induced Urolithiasis (Kant and Singh, 2021).

CONCLUSION

The methanolic extract of *M. uniflorum* seed demonstrated remarkable free radical scavenging efficiency. The horse gram methanol extract effectively scavenged the DPPH and reduced the ferric into ferrous than OH and ABTS free radicals scavenging at high concentrations. Furthermore, the methanolic extract demonstrated considerable anticancer efficiency in a dose-dependent basis on HepG2 cells. Similarly, the horse gram methanol extract has excellent hepatoprotective activity in HEK293 cells against glucose-induced nephrotoxicity. As a result, the results indicate that the methanol extract of horse gram contains pharmaceutically valuable bioactive components. However, more research is needed to conduct in-vivo experiments to assess their pharmacological potential in the near future.

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