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THE BIOMEDICAL EFFICIENCY OF AQUEOUS EXTRACT OF *MACROTYLOMA UNIFLORUM* SEEDS

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Abstract

Plant seeds are the ultimate source of nutrient-dense food, and also pharmaceutically valuable phytochemicals and bioactive components. Thus, this study was designed to evaluate the phytochemical profile, antimicrobial and antioxidant competence of various *Macrotyloma uniflorum* extracts using a standard in-vitro approach. The results obtained from the qualitative phytochemical analysis, revealed that the aqueous extract of *M. uniflorum* seeds contained a significant amount of phytochemicals such as flavonoids, phenols, tannins, saponins, steroids, terpenoids, protein, and carbohydrates. Interestingly, this aqueous extract showed remarkable antimicrobial activity against bacterial (*Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*) and fungal pathogens (*Verticillium* sp. and *Aspergillus flavus*) at more volume (100 µL). Similarly, a dose dependent antioxidant activity was recorded against number of free radicals such as DPPH (93.14%), and phosphomolybdenum (95.42%) by 150.0 µg mL^{-1} concentration. Furthermore, the IC_{50} values of these antioxidant assays were found as 59.75 & 59.77 µg mL^{-1} correspondingly. Thus, obtained results concludes that regular intake of *M. uniflorum* seed-based foods can reduce the deposition of reactive oxygen species and enhance cell viability, resulting in a maximum lifespan. More research is needed to identify the bioactive constituents held to account for antimicrobial and antioxidant activities.

Keywords: *Macrotyloma uniflorum;* Seeds; Phytochemicals; Antimicrobial; Antioxidants.

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Introduction

The constant evolution of habits are a major cause of shortened lifespan in human (Association, 2015). Another of the major causes of ageing process is reactive molecules. In overall, free radicals generated in the human body during energy synthesis method with the utilisation of O_2 molecules (Bhattacharya, 2015). Reactive oxygen species are obtained as a result of mitochondrial ATP synthesis. Moreover, reactive oxygen species are obtained as a result of poor lifestyle choices including such tobacco consumption, environmental degradation, the utilisation of polluted food and drinking water, radioactivity, excessive pharmacotherapy, and so on (Rehman et al., 2018). Surplus deposition of any type of free radical can sometimes be controlled or destroyed in a progressive manner (Di Domenico et al., 2015). Thus overabundance of reactive oxygen species, a cause adverse effects known as oxidative stress created, which is willing to take responsibility for such cell's premature ageing (Hussain and Kayani, 2020).

Moreover, this excessive accumulation contributes to the development of carcinoma, ageing, joint problems, cardiac disease, reactive arthritis, as well as degenerative diseases (Rezuș et al., 2019). Surprisingly, the human body has an intrinsic ability to handle oxidative stress through the production of antioxidant properties as well as the consumption of antioxidant-enriched nutritional supplement (Yamasoba et al., 2013). The antioxidant activities mechanism is insufficient to deal with the surplus reactive oxygen species load (Mohan et al., 2018). Hence, antioxidant-rich feed additives seem to be the most important factor in reducing oxidative stress. Because antioxidant properties can function as oxidative stress foragers or inhibition, they can remedy the situation caused through ROS as well as RNS, potentially improving the immunity and lowering the cancer risk as well as degenerative disease (Lai, 2019).

Because humans live in a contaminated environment, daily ingestion of antioxidant enriched nutrients are more important for maintaining a healthy lifestyle as well as widening one's lifespan (Naik et al., 2021). Nonetheless, the sophisticated and mechanical lifestyle is stealing time from nature's lap. Thus, just one way for ordinary people to consume anti - oxidant agents is in synthetic version (Boppré, 2011). Consequently, discovering antioxidant compounds that are enhanced with potential of being consumed on such a regular basis is critical for improving human health and quality of life (Gulcin, 2020). Several research findings have previously been reported which provide data well about antioxidant activities (Mohamed, 2014) of different plant parts (Grochowski et al., 2017). The *Macrotyloma uniflorum* seeds contains a greater quantity of nutrient enhanced molecules; however, this seed is not preferred by humans as other grains because peoples believe it is only suitable for horses (Kaundal et al., 2019). Nonetheless, the traditional medicinal system employs this seeds as a key component in Ayurvedic medicine preparations for a variety of existence health issues including such asthma, renal release, kidney problems, pneumonia, skin disorders, and cardiovascular ailments (Ambu et al., 2020). Since, the *M. uniflorum* has been enriched with numbers of pharmaceutically valuable phytochemicals such as phenolic acids, flavonoids, tannins, saponins, and so on (Rlds and Erhss, 2017). Hence, this plant seed may possess considerable level of antimicrobial activity against various form of free radicals as well as common microbial pathogens (Mohan et al., 2018). With this perception, this research was framed to evaluate the antioxidant and antimicrobial activity efficiency of *M. uniflorum* seeds through in-vitro approach.

Material and methods

Solvent extraction of seed sample

M. uniflorum seeds were purchased from Krishnagiri District of Tamil Nadu and t he obtained seeds were rinsed with safe drinking water, then dried on a freshly vacuumed floor, and afterwards powdered with such an electrical pulverizer. About 50 g of well powdered *M. uniflorum* seeds was suspended I n 150 mL (each) of hexane, methanol, ethanol, chloroform, and aqueous. The hot extraction method was followed for extraction as well as the excess solvent was evaporated and obtained concentrated each solvent extracts and tested for qualitative phytochemicals, antimicrobial, and antioxidant analyses.

Qualitative analysis of phytochemicals

The pharmaceutically valuable phytoconstituents content of each solvent extract of *M. uniflorum* seeds was investigated using standard protocols. Phytochemicals such as alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids, terpenoids, total proteins, and total carbohydrates were examined using the standard qualitative method.

Antimicrobial activity analysis

The quantifiable phytochemicals analysis study revealed that the aqueous extract alone contains a significant number and volume of phytochemicals compared to other solvent extracts. Hence, the antimicrobial activity of various quantities (40, 60, 80, and 100 µl) of concentrated methanol extract (dissolved in DMSO) of *M. uniflorum* seeds was studied using the standard agar well diffusion method against some common bacterial (*Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*) and fungal pathogens (*Verticillium* sp. and *Aspergillus flavus*) obtained from Microbiology department of Periyar University, Salem. In brief, test microbial pathogens were inoculated on sterile Mueller Hinton Agar (MHA) plates (in triplicates) using the spread plate method, and then various volumes (40, 60, 80, and 100 µl) of concentrated methanol extract dissolved in DMSO were poured onto perforated MHA plate wells. The bacterial and fungal pathogens inoculated plates were incubated at 35˚C for 24 h and 28˚C for three days, respectively. Amoxicillin (30 µg mL^{-1}) and Fluconazole (60 µg mL^{-1}) were used as positive controls for antibacterial and antifungal activity. After incubation, the zone of inhibition was measured and compared to a positive control.

Free radicals scavenging potential analysis

The antioxidant reducing potential of various concentration of (25 to 150 µg mL^{-1}) of aqueous extract of *M. uniflorum* seeds were studied through in-vitro approach using different form of radicals that including DPPH (2,2- diphenyl-1-picrylhydrazyl), H_2O_2, potassium ferricyanide (FRAP), and phosphomolybdenum.

DPPH assay

The standard protocol was followed to evaluate the DPPH radical scavenging study of *M. uniflorum* seeds. Around 20 µL of the above mentioned aqueous extract concentrations were mixed with 80 µL of 100 mM of DPPH, and vitamin C was applied as a positive control. Later again incubated in the shadows for few minutes at 37 °C. The sample's absorbance was then measured at 517 nm with a UV-vis spectrophotometer. The IC_{50} value as well as free radicals scavenging percentage were calculated subsequently.

H_2O_2 radical scavenging activity

The H_2O_2 free radicals scavenging potential of the above-mentioned dosage of aqueous extract of *M. uniflorum* seed was analysed through the standard H_2O_2 scavenging protocol. In brief, about 0.2 mL of EDTA, $FeCl_3$, and 2-deoxy-d-ribose as well as 0.8 mL of phosphate

buffer was mixed together. Subsequently the mixer was kept in boiling water bath for few minutes, later 0.2 mL of vitamin C and H_2O_2 were added and incubated for 1 h at 37 °C. After which 1.5 mL of (each) ice cold thiobarbituric acid and HCl (25%) were augmented and then boiled at 100 ℃ for 15 min then warmed at ambient heat. Furthermore, catechin was utilized as a positive control, as well as then the absorbance of test and standard was measured at 532 nm with a UV-vis spectrophotometer. The H_2O_2 radical scavenging percentage and IC_{50} value were calculated using standard formula.

Potassium ferricyanide reducing assay

Accordance with the standard methodology, the $K_3[Fe(CN)_6]$ reducing efficacy of various dosages of aqueous extract of *M. uniflorum* seeds was determined. Concisely, 0.6 mL of individual dosage of test samples were mixed with 2.4 mL of $K_3[Fe(CN)_6]$ dispersed in a potassium buffer (0.2 M). The samples were incubated for 20 minutes at 50 °C before being centrifuged at 8000 rpm for 10 minutes with 0.5 mL of 10% TCA to stop the reaction. The filtrates (1.8 mL) was mixed with 0.2 ml distilled water, as well as 0.1 percent $FeCl_3$ reagent, as well as the resulting blend was recorded at 700 nm using a UV-vis spectrophotometer. In addition, the IC_{50} value of methanolic extract of *M. uniflorum* seeds was measured at 593 nm with a UV-vis spectrophotometer and compared to quercetin as standard (25 to 150 µg/ml).

Phosphomolybdenum (PM) assay

The entire free radicals scavenging activity of aqueous extracts of *M. uniflorum* seeds was investigated using the standard phosphomolybdenum assay as described by Murugan and Parimelazhagan (Murugan and Parimelazhagan, 2014). In brief, the above mentioned concentration of methanolic extract of *M. uniflorum* seeds were mixed with 3 mL of distilled water and subsequently added 1 mL of Molybdate reagent. These reaction mixtures were incubated at 95˚C for 90 min. After incubation the absorbance was measured at 695 nm through UV-vis spectrophotometer. Similar concentration of vitamin C was used as a positive control. The free radicals scavenging percentage and IC_{50} were calculated using standard formulas.

Results and discussion

The qualitative analysis of phytochemicals

The *M. uniflorum* seeds has been used as an energy supplements due to its enriched nutritional value. The qualitative phytochemical analysis revealed that the aqueous extract of *M. uniflorum* seeds contains a significant number of pharmaceutically valuable phytochemicals such as flavonoids, phenols, tannins, saponins, steroids, terpenoids, protein, and carbohydrates. It was followed by methanol extract (flavonoids, phenols, tannins, saponins, steroids, terpenoids, protein, and carbohydrates) and ethanol extract (alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids, and terpenoids) (Table 1). These results were correlated with the findings of Kaundal et al., they reported that seeds of *M. uniflorum* contains pharmaceutically valuable phytochemicals such as flavonoids, terpenoids, tannins, alkaloids, phenols, and so on (Kaundal et al., 2019). The other parts of *M. uniflorum* also were reported by researchers as they contain significant quantity of biomedical valued phytochemicals (Chakraborty et al., 2018; Suriyamoorthy et al., 2014). These individual phytochemicals possess unique biomedical potentials. Numerous flavonoids have been shown as possess free radicals scavenging activity, cardiovascular disease protection, hepatoprotective, anti-inflammatory, as well as anti-carcinogenic properties, while others have considerable antiviral properties (Kumar and Pandey, 2013).

Terpenes have a wide range of medicinal applications, including antiplasmodial, antiviral, anticancer, and antidiabetic reagents (Cox-Georgian et al., 2019). Tannins have medical uses due to their slightly bitter attributes. They encourage healing process and also the growth of new body tissue on acute inflammatory mucous membranes and lacerations. Tannins compounds are being used to cure varicose ulcers, haemorrhoids, chemical burns, frostbite, and gum inflammation (Sagbo et al., 2017). Saponins reduced blood lipids, reduce risks of cancer, and improve blood sugar response. An elevated saponin nutrition is being used to prevent dental cavities and platelet activation, cure hypercalciuria in individuals, and act as such an effective remedy to acute heavy metal poisoning (Shi et al., 2004).

Antimicrobial activity competence

The qualitative phytochemical analysis results revealed that the aqueous extract showed considerable number of phytochemicals than other extracts. Hence, the various concentrations (40, 60, 80, and 100 µl) of aqueous extract were tested against common bacterial (*K. pneumoniae, S. aureus*, and *E. coli*) and fungal pathogens (*Verticillium* sp. and *A. flavus*) and fungal pathogens through agar well diffusion method. The results obtained from this study revealed that the both antibacterial and antifungal activities were dose dependent. Since at increased concentration, the zone of inhibition of aqueous extract of *M. uniflorum* seeds against the test bacterial pathogens were found as for *E.coli* (0.9 to 7 mm), *S. aureus* (2.0 to 5.0 mm), and *K. pneumoniae* (3.0 to 6.0 mm) at the volume ranging from 40 to 100 µL. Similarly, the zone of inhibition for fungal species *A. flavus* and *Verticillium* sp were found as 0.9 to 1.6 mm and 0.7 to 1.5 mm. These zone of inhibition range of aqueous extract of *M. uniflorum* seeds against bacterial and fungal pathogens were partially comparable with the zone of inhibition of positive control (Table 2). These results were partially discussable with the report of Mr and Kr (2015), they reported that the *M. uniflorum* alcohol extract showed considerable antibacterial activity against many bacterial pathogens such as *P. aeruginosa, P. argentinensis, E. coli, B. subtilis, V. mimicus, K. pneumonia, S. paratyphi, V. harveyi* and *Pseudomonas* sp at the volume of 50 to 100µl (Mr and Kr, 2015). Similarly, another report stated that the various extracts and various parts of *M. uniflorum* possess considerable volume of pharmaceutically valuable phytochemicals (Chakravarty et al., 2019). According to this, in the present study the aqueous extract which applied for antimicrobial activity had shown considerable number of pharmaceutically valuable phytochemicals such as lavonoids, phenols, tannins, saponins, steroids, and terpenoids. Hence, these phytochemicals have been previously reported as possess remarkable antimicrobial activity against test bacterial and fungal pathogens. Similarly, another report stated that the aqueous crude extract of *M. uniflorum* possess remarkable antifungal activity against most common fungal pathogens such as *A. alternate, F. equiseti, C. lunata, M. Phaseolina, C. corchori*, and *B. theobromae* (Kawsar et al., 2008).

Free radicals scavenging potential analysis

DPPH assay

The DPPH free radical scavenging efficiency of *M. uniflorum* seeds aqueous extract was found to be very high. Furthermore, the DPPH scavenging is considered the best criterion in assessing anti - oxidant ability. The proportion of DPPH radical scavenging activity of *M. uniflorum* seeds aqueous extract at dosage of 25 to 150 µg mL^{-1} was found to be 71.23 to 93.14% in this study and it was statistical significance at $P>0.03$ (Fig. 1a). Similarly, an aqueous extract of *Foeniculum vulgare* was found to scavenge 96.2% of DPPH radicals.

Besides that, the IC_{50} of aqueous extract on DPPH was determined to be 59.75 µg mL^{-1}, which was similar to positive control ascorbic acid (55.86 µg mL^{-1}) (Fig. 1b). Because it has a low IC_{50} value of 0.95 µg mL^{-1}, it suggests that the aqueous extract of *M. uniflorum* seeds has higher DPPH scavenging activity than graph seeds (Čanadanović-Brunet et al., 2009). The aqueous extract of *M. uniflorum* seeds may contain considerable amount of DPPH scavenging compounds, that act as hydrogen atom donors (Amarowicz et al., 2000). Thus can prevent the activity of oxidants (Goswami et al. 2014). Interestingly, Siddhuraju and Manian, reported that various solvent extracts of *M. uniflorum* showed considerable DPPH radicals scavenging activity at 600 µg mL^{-1} concentration (Siddhuraju and Manian, 2007).

H_2O_2 radicals assay

In this study, the lowest antioxidant activity was observed as 58.89 ±1.2% (Fig. 2a) at 150 µg mL^{-1} concentration, which was found as not significant. At a dosage of 150 µg mL^{-1}, the catechin demonstrated 96.35 % scavenging activity. Correspondingly, the poor IC_{50} value seemed to be 111.60 ± 1.5 µg mL^{-1} (Fig. 2b) rather than the control (66.01 ± 1.7 µg mL^{-1}). Additional H_2O_2 abundance can prompt lipid peroxidation as well as harm to lipid membranes (Uttara et al., 2009). The hydroxyl radical may arise from H_2O_2 as well as super oxide in the existence of metals (Cu^{2+} and Fe^{2+}). Furthermore, hydroxyl radicals can interfere with nucleotides and induce DNA strand damage, resulting in mutations, malignancies, and cytotoxicity (Rahal et al., 2014). *M. uniflorum* seeds aqueous extract contains H_2O_2 radical scavengers. It could be similar to polyphenolic compounds, that can connect and deactivates oxidants while also repairing the damage induced by hydroxyl radicals (Goswami and Chatterjee, 2014).

Ferriccyanide reducing potential analyses

In this research, the reduction of ferricyanide to ferrocyanide was demonstrated with standard protocol. This was approximately equal towards the increasing amount of *M. uniflorum* seeds aqueous extract, i.e., dose depended basis. Remarkably, a 98% decrease in ferricyanide was observed on 150 µg mL^{-1} dosage of aqueous extract, which was almost close to positive control (quercetin) (Fig. 3a). The IC_{50} of aqueous extract of *M. uniflorum* seeds was determined to be 64.85± 2.7 µg mL^{-1}, which was comparable with positive control as 60.19 ± 2.3 µg mL^{-1} (Fig. 3b). According to the findings, the aqueous extract of *M. uniflorum* seeds contains ferricyanide reducing phytochemicals. Correspondingly, pomegranate seed extracts have outstanding ferric reducing potential. (Basiri, 2015). The conversion of ferric to ferrous as well as Fe(III) to Fe(II) suggests that it may contain so much phenolic constituents (Siddhuraju and Becker, 2003). The reduction was accomplished through the transfer of electrons/hydrogens from antioxidant compounds to oxidizing agents. The great decrease may emerge at acidic (3 to 5) pH levels. It keeps ions soluble, which enhances the process (Prior et al., 2005). Oxidizing agents, usually the polyphenolic compounds found in extracts, may reduce the Fe^{3+} - Fe^{2+} ratio that may be noteworthy. Concurrently, the existence of reduction in the occurrence of trypyridyl-s-triazine may result in the formation of coloured complexes such as Prussian blue. (Shahidi and Zhong, 2015). Similarly, Siddhuraju et al. (Siddhuraju and Becker, 2003), reported that elevated / low molecular mass polyphenol constituents from various solvent extracts of groundnut husk as well as Indian laburnum stem bark extract were shown to have strong antioxidant properties. As a result, the obtained findings confirm that the aqueous extract of *M. uniflorum* seeds may

contain a significant volume of reductants in the type of phenolics (Amarowicz et al., 2000).

Phosphomolybdenum antioxidant analysis

The *M. uniflorum* seeds aqueous extract exhibited substantial phosphomolybdenum reducing activity ranging from 65.27 ± 1.9 - 95.42 ± 2.7 % at dosages ranging from 25 - 150 µg mL^{-1} (Fig. 4a). Furthermore, the reducing activity found at 150 µg mL^{-1} that was almost compared to ascorbic acid scavenging potential (99.41%). The IC_{50} of aqueous extract was determined to be 59.77 ± 1.4 µg mL^{-1} (Fig. 4b), which was significant compared to the positive control (56.0 ± 2.3 µg mL^{-1}). The phenolic as well as flavonoid content can reduce the phosphomolybdenum, rendering it inactive. Likewise, Jan et al. discovered that perhaps polyphenols as well as flavonoids compounds in *M. buxifolia* have substantial phosphomolybdenum reducing potency (Jan et al., 2013). Hence, regular intake of antioxidant-rich foods may help to significantly reduce the hydroxyl radical deposition and assist the growth and maintenance of cell with improved metabolism (Nazir et al., 2019).

Conclusions

The results obtained from this study revealed that the aqueous extract of *M. uniflorum* seeds showed that the aqueous extract contained considerable number of pharmaceutically valuable phytochemicals. Furthermore, the aqueous extract showed considerable antimicrobial activity against most common bacterial and fungal pathogens at increased volume (100 µL). Moreover, this aqueous extract also showed remarkable free radicals scavenging activity at increased concentration (150 µg mL^{-1}), as well as it was interestingly close to the standard antioxidant agent's activity. Thus, these study concludes and recommend that regular intake of *M. uniflorum* seed-based foods could reduce the deposition of reactive oxygen species and enhance cell viability, resulting in a maximum lifespan. More research is needed to identify the bioactive constituents held to account for antimicrobial and antioxidant activities.

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Figure legend:

Figure 1 Percentage of DPPH free radical scavenging activity of methanol extract

(a): Percentage of DPPH radical scavenging activity **(b):** IC_{50} value of methanol extract in DPPH radical scavenging activity. ***:** represents statistically significant at $P< 0.03$

Figure 2. Percentage of hydroxyl radical scavenging activity of methanol extract

(a): Percentage of hydroxyl radical scavenging activity **(b):** IC_{50} value of methanol extract in hydroxyl radical scavenging activity. ***ns:*** represents statistically not significant.

Figure 3. Percentage of ferricyanide reducing competence of methanol extract

(a): Percentage of ferricyanide reducing antioxidant activity **(b):** IC_{50} value of methanol extract in ferricyanide reducing antioxidant activity. ***:** represents statistically significant at $P< 0.03$

Figure 4. Percentage of phosphomolybdenum radical scavenging activity of methanol extract

(a): Percentage of phosphomolybdenum radical scavenging activity **(b):** IC_{50} value of methanol extract in phosphomolybdenum radical scavenging activity. ***:** Represents statistically significant at $P< 0.03$

Figure 1.

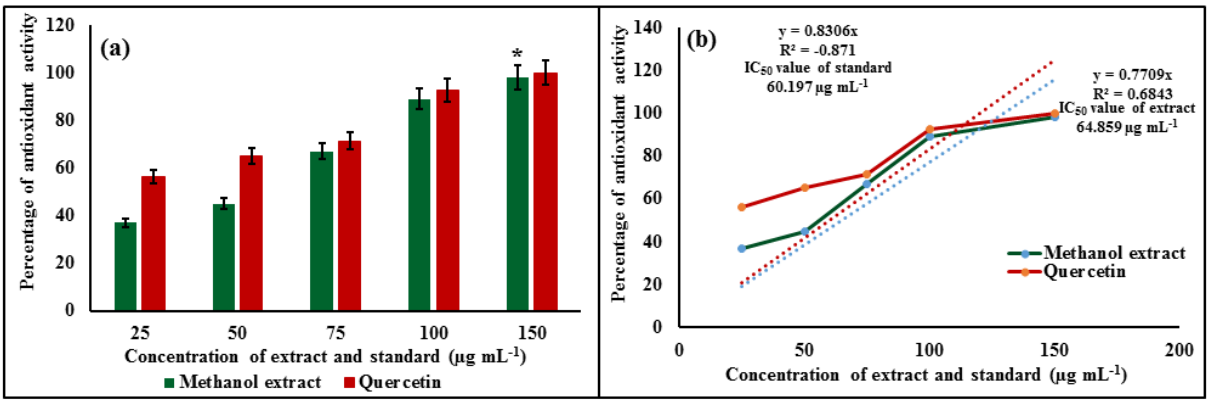

Figure 2.

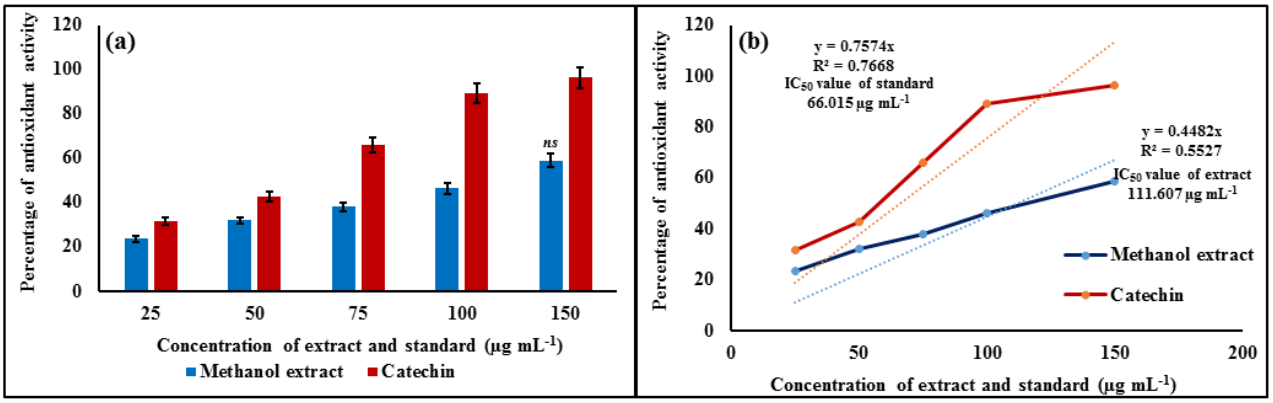

Figure 3.

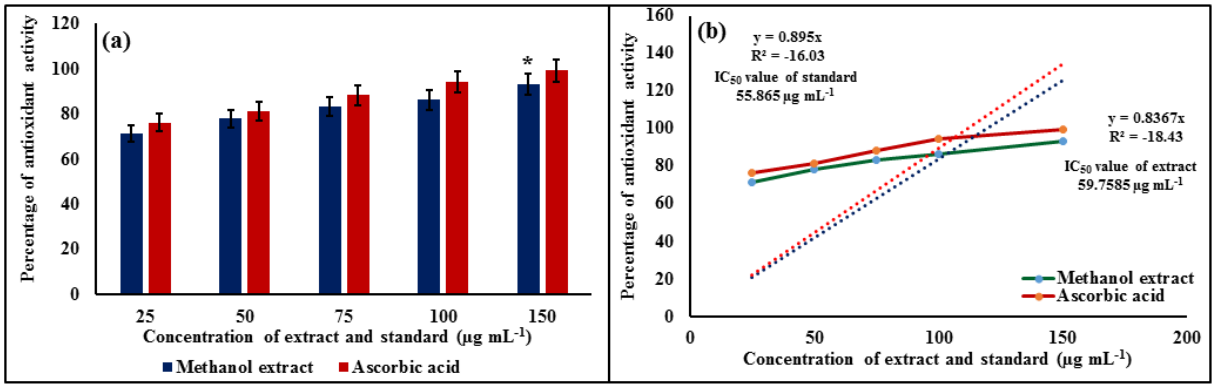

Figure 4.

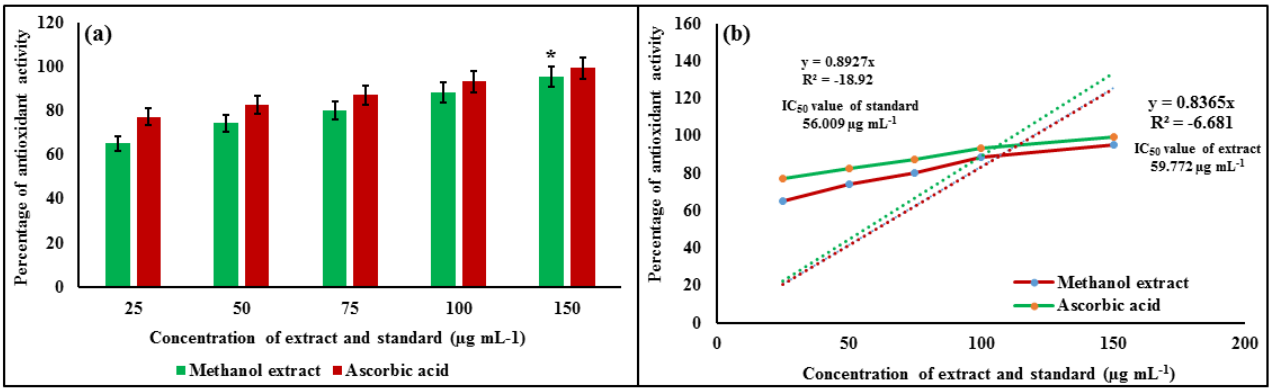

Table 1: Qualitative phytochemical screening of *M. uniflorum* seeds

S. No	Phytochemical Constituents	Various solvent extracts				
		Hexane	Chloroform	Methanol	Ethanol	Aqueous
1	**Alkaloids**	-	-	-	+	-
2	**Flavanoids**	-	-	+	+	+++
3	**Phenols**	-	-	+	+	++
4	**Tannins**	-	-	+	+	++
5	**Saponins**	-	-	+	+	+++
6	**Glycosides**	-	-	-	+	-
7	**Steroids**	-	-	+	+	++
8	**Terpenoids**	-	-	+	+	+
9	**Proteins**	-	+	+	-	+++
10	**Carbohydrates**	-	+	+	-	++

Legend: +++: Strongly present, **++:** Moderately present; **+:** Present; **-:** Absent

Table 2: Antimicrobial activity of aqueous extract of *M. uniflorum* seeds

Extract	Microbes	Volume of Sample (µL)	Zone of Inhibition (mm)
Bacterial pathogens	*E.coli*	40	0.9 ± 0.3
		60	3 ± 0.2
		80	5 ± 0.4
		100	7 ± 0.3
	S. aureus	40	2 ± 0.4
		60	4 ± 0.2
		80	5 ± 0.6
		100	5 ± 0.4
	K. pneumoniae	40	3 ± 0.3
		60	4 ± 0.3
		80	5 ± 0.4
		100	6 ± 0.3
	Amoxicillin (µg mL^{-1})	30	10 ± 0.2
Fungal pathogens	*A. flavus*	40	0.9 ± 0.3
		60	1.1 ± 0.2
		80	1.4 ± 0.4
		100	1.6 ± 0.3
	Verticillium sp.	40	0.7 ± 0.3
		60	1.0 ± 0.5
		80	1.1 ± 0.4
		100	1.5 ± 0.6
	Fluconazole (µg mL^{-1})	60	1.9 mm - 2.4 mm

Legend: The mentioned values are mean and standard error (±SE) of triplicates.

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