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Cell damage preventing potential and antimicrobial activities of *Pandanus tectorius* leaves extract

T Ramesh[1*], A Amuthavalli[2]
[1] Department of Insect-Plant Interaction, G.S. Gill Research Institute, A Unit of Guru Nanak Educational Society, Affiliated to University of Madras, Guru Nanak Salai, Velachery, Chennai, Tamil Nadu, India
[2] Department of Microbiology, Dr. ALM PGIBMS, University of Madras, Taramani Campus, Chennai, Tamil Nadu, India

Abstract
Free radicals are unstable molecules missing an electron and can damage other molecules by taking electrons from them. This damage can affect intracellular components, potentially causing cell death. These oxygen-based molecules can harm cells by damaging lipids, proteins, and DNA. ROS can be produced both within the body and from external sources, including pollution, cigarette smoke, UV radiation, and heavy metals. When free radicals are produced faster than the body's antioxidant defenses can handle, oxidative stress occurs, leading to damage in cellular structures such as membranes, proteins, and DNA. Oxidative stress is linked to numerous diseases, including cancer, asthma, pulmonary hypertension, and retinopathy. Consequences of oxidative stress may be reduced with aid of antioxidants, which neutralize free radicals and stop more oxidation processes. Enzymatic and non-enzymatic elements make up antioxidant system in body. Non-enzymatic molecules from plant source act in various ways, such as inhibiting enzymes, binding trace elements that contribute to free radical production, and boosting other antioxidant defenses. Among these molecules, alkaloids derived from secondary metabolism play a crucial role in combating oxidative stress. These compounds act as antioxidants not only because they donate electrons or hydrogen but also because they are stable in their radical intermediate form. Therefore, studying the ability of methanolic extracts from *Pandanus tectorius* to scavenge free radicals and reduce ROS could help assess the potential of these plants as sources of novel antioxidant compounds.

Keywords: *P. tectorius*, free radicals, antioxidant, cell damage prevention, antimicrobial activity

Introduction
Since ancient times, people have used plant and animal products for their therapeutic properties, often through simple methods that didn't require isolating pure compounds. The pharmacological effects of these natural drugs depend on the nature of their components. Some examples of substances found in plants and their physiological effects include alkaloids, terpenoids, flavonoids, glycosides and phenolics. Plants' secondary metabolites that contain various chemicals and show promise in biological activities are mostly responsible for therapeutic advantages. (Dias *et al*., 2012) [8].

Pandanus tectorius, commonly known as Thazham poo chedi, belongs to the Pandanaceae family, comprises over 700 species prevalent in tropical and subtropical areas, such as Indonesia and Malaysia (Anirudhan *et al*., 2023) [4]. Ayurvedic practitioners in India have long relied on *P. tectorius* leaves for a variety of medical conditions. Chemical make-up is a strong predictor of a substance's medicinal potential. According to Kholieqoh *et al*., (2024) [14], traditional medicine has long relied on pandanus leaf oil to treat a variety of ailments, including earaches, headaches, arthritis, exhaustion, vertigo, constipation, rheumatism, smallpox, and muscular spasms. There are a lot of secondary metabolites in its leaves.

Plant secondary metabolites help neutralize excess reactive oxygen in the body, which protects cells from damage and lowers the chances of diseases (Kholieqoh *et al*., 2024) [14]. Additionally, phytochemicals can influence oxidative stress-related signaling, potentially preventing cellular transformations that lead to cancer. Therefore, plants have remained a crucial source of medicine throughout history (Raj *et al*., 2014) [21]. Hence, main aim of our research was analyzing protective potential of the methanolic extract of *Pandanus tectorius* leaves against oxidative stress and free radicals. The study also aimed to explore the antimicrobial activities of this extract.

Materials & Methods
Collection of plant sample
The leaves of *Pandanus tectorius* were collected from Tholkappia Poonga (Adyar Eco Park), Chennai.

Processing of plant sample
Leaves subjected to thorough washing process, using tap water twice and sterile water once. After washing, the *P. tectorius* leaves were dried on a cotton cloth. The leaves were then placed in a hot air oven at 45°C for 4 days. After 4 days, the dried leaves were then ground and weighed again. The recorded values were noted, and the ground leaves were prepared for further analysis (Dubale *et al*., 2023) [9].

Preparation of extract
A 50 g sample was weighed and soaked in 250 ml of methanol. The extract was left to stand overnight, then filtered via sterile filter paper. Filtrate was gathered, measured, and stored in a beaker for further analysis (Chaves *et al*., 2020) [6].

Phytochemical analysis
Test for alkaloids
A little amount of Dragendorff's reagent was added to 1 millilitre of sample, and mixture was then watched for an orange-red colour (Kancherla *et al*., 2019) [13].

Test for saponins
2 ml of H_2O added to 1ml sample followed by vigorous shaking and appearance of foam confirm the presence of saponin (Auwal *et al*., 2014) [5].

Test for cardiac glycosides
1ml of extract mixed with 0.4ml of glacial acetic acid, ferric chloride solution and concentrated H_2SO_4 produce a violet ring beneath the brown ring formation (Pant *et al*., 2017) [19].

Test for proteins
A few drops of Bradford reagent added to 1ml sample and the development of blue colour confirm the presence of protein (Ernst & Zor, 2010) [10].

Thin Layer Chromatography
TLC was performed to isolate the primary components present in the most effective plant extracts using appropriate solvent systems. Mobile phase consisted of 6:4 ratio of methanol and ethyl acetate, whereas stationary phase consisted of pre-coated TLC plate. TLC was performed by the procedure outlined by Kowalska & Sajewicz, (2022) [15], retention factor (Rf) values were computed by monitoring migration of separated bands.

Reducing power activity
The presence of reductones in an extract is typically linked to its reducing properties, as these compounds can disrupt free radical chains by donating hydrogen atoms. An increase in absorbance reflects a rise in the extract's reducing power and antioxidant activity. The extract's absorption is deliberated at 700 nm relative to blank solution (Yen & Duh, 1993) [25].

***In vitro* superoxide dismutase**
Superoxide dismutases (SODs) are enzymes that transform superoxide into hydrogen peroxide and oxygen, serving as the primary defense against oxygen free radicals. They regulate the levels of ROS and RNS. In laboratory settings, superoxide dismutase (SOD) activity is assessed to find a new nanozymes and predict their possible biological impacts. The SOD assay was performed based on the method described by Alici *et al*., (2016) [3], with absorbance measured at 560 nm.

Hydrogen peroxide scavenging assay
Hydrogen peroxide (H_2O_2) is a reactive oxygen species that, at high concentrations, can be damaging to cells. A hydrogen peroxide scavenging assay assesses a substance's capacity to neutralize hydrogen peroxide. The scavenging ability of an extract had been analyzed with technique described by Al-Amiery *et al*., (2015) [1], with absorbance measured at 560 nm.

Antimicrobial activity
Agar well diffusion technique was used to assess antibacterial properties of a sample by using Muller Hinton Agar (MHA) medium. A cork borer was used to make wells, and then sample and positive control (Streptomycin, 1mg/ml-20 µl) were added to each well. Next step was to incubate plates at 37ºC for 24 hrs. The diameter of inhibitory zone was measured after incubation. (Valgas *et al*., 2007) [23].

Well diffusion on Potato Dextrose Agar (PDA) medium was used to assess sample's antifungal activity. A cork borer was used to create wells, and then sample and positive control (Ketoconazole 1mg/ml-20µl) were added to the wells. At 37ºC, the plates were left to incubate for 24 hrs. The zone of inhibition was measured and documented. (Ramani & Chaturvedi, 2000) [22].

Results

Preparation of extract
The crude extract obtained after filtration had a final volume of 98 ml. This extract was used for phytochemical and TLC analysis, while the solvent-free extract was employed in other assays conducted in this study (Figure 1).

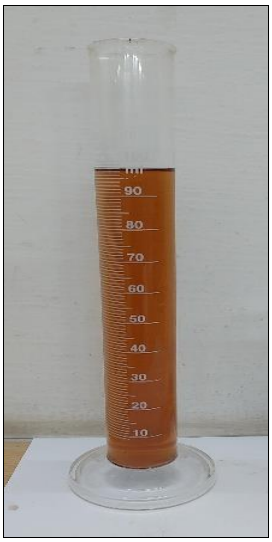


Fig 1: Phytochemical extract

Phytochemical analysis
Bioactive compounds are natural chemicals found in plants and some foods that may support overall health. They are being researched for their potential to prevent and treat various diseases. Phytochemical analysis helps identify the specific bioactive compounds in plants (Figure 2 & Table 1). The qualitative analysis of the present study results confirms the presence of alkaloid and absence of diterpenes, saponins, cardiac glycosides and proteins.

Fig 2: Phytochemical analysis result of alkaloid

Table 1: Table showing the phytochemical analysis results of extract

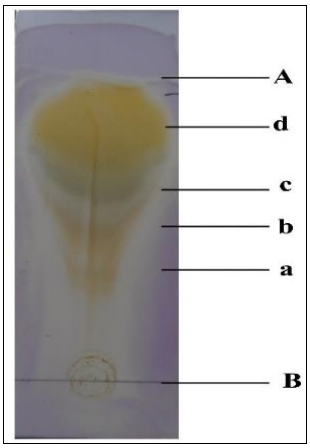
Phytochemicals	Results
Alkaloids	+
Diterpenes	-
Saponins	-
Cardiac glycosides	-
Proteins	-

Thin Layer Chromatography

Thin Layer Chromatography is employed to separate the components of secondary metabolites in plant extracts. The results revealed a notable diversity of compounds separated from the secondary metabolites. The TLC profile of the crude extract in Table 2 displays four distinct bands (Figure 3). The alkaloids are identified by Rf values of 0.32, 0.49, and 0.60, while tannins are confirmed by an Rf value of 0.76.

Table 2: Table showing the TLC results of extract

S. No.	Sample fractions	Distance moved by the solvent (A) (cm)	Distance moved by the solutes (cm)	R_f (b/A)
1.	a	5.5	1.8	0.32
2.	b	5.5	2.7	0.49
3.	c	5.5	3.3	0.60
4.	d	5.5	4.2	0.76



Sample moved = A to D / Solvent moved = A / Sample spot = B

Fig 3: TLC profile of plant extract

Reducing power activity

The reducing power of *P. tectorius* extract was evaluated in this study, and it was found that the 100-µg concentration sample showed 0.64% inhibition compared to the standard. Furthermore, the percentage of inhibition increasing while increasing the concentration of extract (Figure 4 & Table 3).

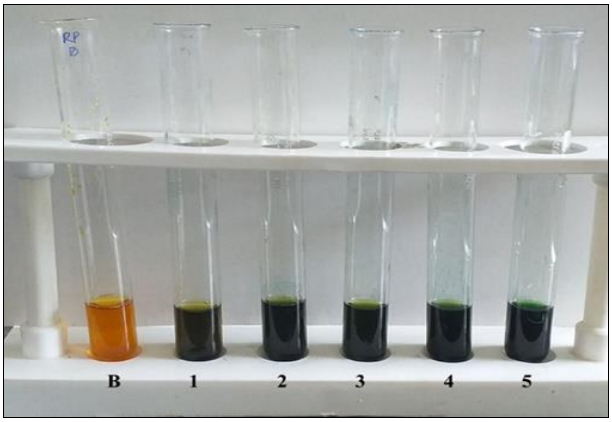


Fig 4: Results of reducing power activity of extract

Table 3: Reducing power activity of the extract

Sample / Con. (µg)	100	200	300	400	500
% inhibition	0.64	0.73	0.81	0.89	0.98
Ascorbic acid Con. (µg)	**100**	**200**	**300**	**400**	**500**
% inhibition	0.40	0.75	1.21	1.60	1.97

In vitro superoxide dismutase

Superoxide dismutase (SOD) enzyme activity result was revealing that the extracts exhibited minimum SOD activity, with values between 14% and 30% (Figure 5 & Table 4). Higher concentrations of the sample showed no SOD enzyme activity.

Fig 5: *In vitro* superoxide dismutase activity

Table 4: *In vitro* superoxide dismutase activity

Sample Con. (µg)	100	200	300	400	500
% inhibition	30.4	15.2	14.3	-	-
Ascorbic acid Con. (µg)	**100**	**200**	**300**	**400**	**500**
% inhibition	52.0	42.4	23.2	12.3	-

Hydrogen peroxide scavenging assay

Hydrogen peroxide is typically found in low concentrations in various environments, such as the air, water, human body, plants, microorganisms, and food. Hydroxyl radicals (OH·) may be produced during its rapid breakdown into oxygen and water, which might lead to DNA damage and lipid peroxidation. Due to the presence of alkaloids, that may contribute electrons and convert hydrogen peroxide into water, methanolic extract of *P. tectorius* leaf demonstrated a moderate ability to scavenge hydrogen peroxide (Figure 6 & Table 5).

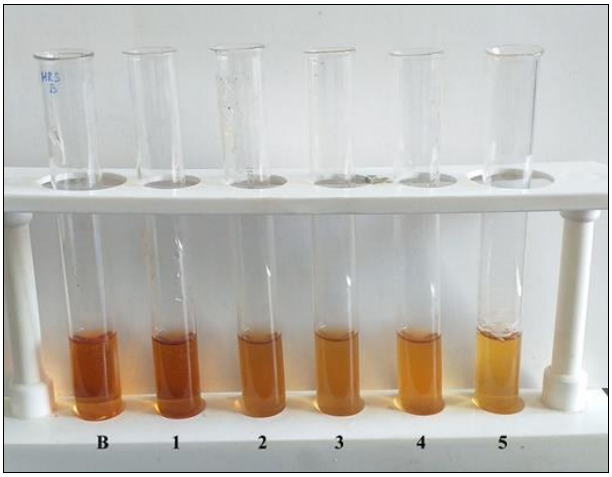


Fig 6: Hydrogen peroxide scavenging assay

Table 5: Hydrogen peroxide scavenging assay

Sample Con. (µg)	100	200	300	400	500
% inhibition	5.5	16.6	25.0	33.3	44.4
Ascorbic acid Con. (µg)	**100**	**200**	**300**	**400**	**500**
% inhibition	22.5	30.0	37.5	60.0	80.0

Antimicrobial activity

This study evaluated the antibacterial effects of *P. tectorius* leaf extract on *Staphylococcus aureus*. The 60 µl and 80 µl extracts demonstrated inhibitory activity, while other concentrations showed no effect against *S. aureus* (Figure 7 & Table 6).

In this study, the antifungal activity of *P. tectorius* leaf extract against *Aspergillus niger* was examined. No inhibition was noted at any of the concentrations, other than the positive control (Figure 8 & Table 6).

Table 6: Antimicrobial activity results of plant extract

	Zone of Inhibition in mm				
Microorganisms	**20 µl**	**40 µl**	**60 µl**	**80 µl**	**Control**
Bacteria					
Staphylococcus aureus	-	-	8	10	28
Fungi					
Aspergillus niger	-	-	-	-	33

Controls: Streptomycin (Bacteria) & Ketoconazole (Fungi)

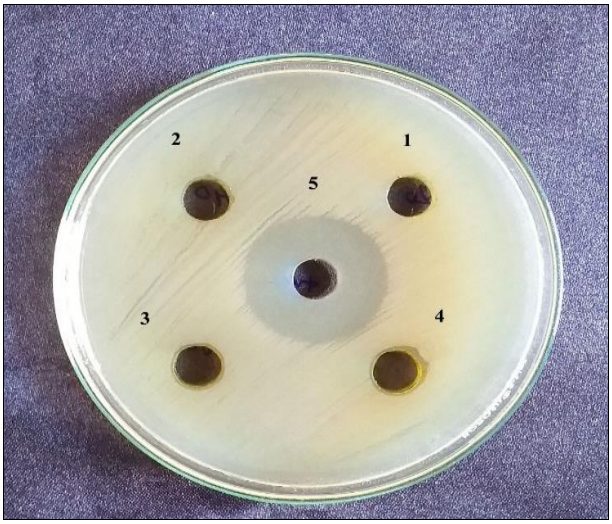


Figure 7: Antibacterial activity against *S. aureus*

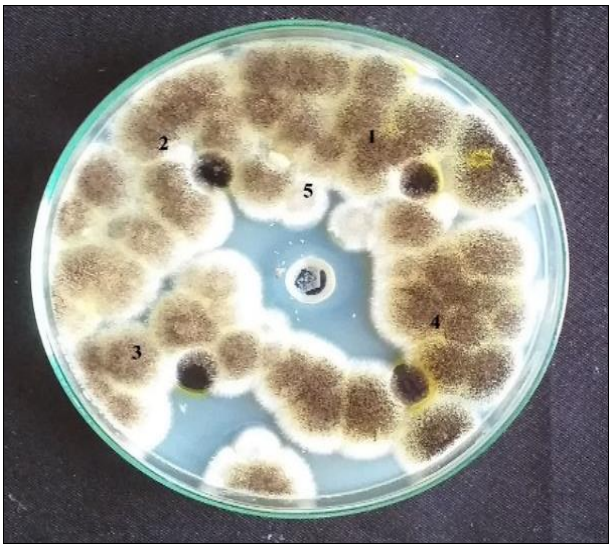


Fig 8: Antifungal activity against *A. niger*

Discussion

Methanolic extracts of *P. tectorius* leaves were analyzed for phytochemical content, revealing the presence of alkaloids. According to Kumar and Sanjeeva (2011) [16], reported that Pandanus plant parts contain phenols, tannins, steroids, carbs, proteins, and alkaloids and it was discovered that these phytochemical features were linked to plant's bioactivity. According to many researchers (Dahija *et al*., 2014; Mohammed *et al*., 2014; Lunga *et al*., 2014; Ali *et al*., 2002) [2, 7, 17, 18], phytochemical components possess antioxidant and antibacterial properties. One typical way to test an antioxidant's capacity to donate electrons is using reducing power assay. A test sample colour changes from green to blue in reducing power test, which measures the antioxidants' ability to decrease Fe^{3+} to Fe^{2+} (Ferreira *et al*., 2007) [11]. Table 3 shows the reducing activities of *P. tectorius* leaf extract when compared to ascorbic acid. A higher absorbance indicates a greater reducing power. The superoxide dismutase (SOD) activity was examined in this study demonstrated that the extract exhibited negligible levels of SOD activity. The role of SOD is to convert superoxide radicals into hydrogen peroxide and oxygen. While superoxide radicals are produced throughout aerobic metabolism, they are not directly harmful to DNA. However, they can generate hydroxyl radicals that cause DNA damage (Ighodaro & Akinloye, 2018) [12]. In addition, prooxidative transition metal ions can catalyze the formation of DNA oxidants, leading to further DNA damage. Superoxide also affects lipid membranes by releasing lipid radicals, which can trigger lipid peroxidation. Thus, SOD is important for maintaining cellular antioxidant defenses (Zheng *et al*., 2023) [26]. The methanolic extract exhibited a moderate ability to neutralize hydrogen peroxide, likely due to the alkaloids that can donate electrons and reduce hydrogen peroxide to water. Hydrogen peroxide is known to cause DNA damage, including strand breaks and cross-links, as well as modifications to pyrimidines, purines, and deoxyribose (Valverde *et al*., 2018) [24]. In Fenton reactions, Fe2+ ions from transition metals donate electrons to hydrogen peroxide, breaking it down and producing hydroxyl radicals. These radicals can then interact with DNA, lipids, and other cellular components, leading to oxidative damage (Pizzino *et al*., 2017) [20]. In this study, *Staphylococcus aureus* served as a model for pathogenic bacteria, while *Aspergillus niger* represented fungi, to assess antimicrobial properties. The 60 µl and 80 µl concentrations of the extract showed inhibition (10 mm), but other concentrations had no effect on *S. aureus*. But none of the sample concentration did not show any activity against *A. niger*. This research enhances our understanding of the antimicrobial mechanisms of *P. tectorius* leaf extract and paves the way for future work focused on isolating and identifying active antimicrobial compounds.

Conclusion

This research focuses on the *in vitro* antioxidant properties of the methanolic leaf extract of *Pandanus tectorius*, including its reducing power, superoxide dismutase activity, and ability to scavenge hydrogen peroxide, which may help protect cells from oxidative stress and free radical damage. The extract's potential to combat oxidative stress underscores its promise as a novel drug candidate. Furthermore, the extract's antibacterial effects may lead to the development of effective alternatives to traditional antibacterial treatments.

References

1. Al-Amiery AA, Al-Majedy YK, Kadhum AA, Mohamad AB. Hydrogen Peroxide Scavenging Activity of Novel Coumarins Synthesized Using Different Approaches. PLoS One,2015:10(7):e0132175.
2. Ali MS, Saleem M, Yamdagni R, Ali MA. Steroid and antibacterial steroidal glycosides from marine green alga Codium iyengarii Borgesen. Nat Prod Lett,2002:16(6):407-13.
3. Alici EH, Arabaci G. PPO and CAT Enzyme Activities in Rumex Obtusifolius L. Ann Res Rev Biol,2016:11(3):1-7.
4. Anirudhan A, Iryani MTM, Andriani Y, Sorgeloos P, Tan MP, Wong LL, *et al*. The effects of *Pandanus tectorius* leaf extract on the resistance of White-leg shrimp Penaeus vannamei towards pathogenic Vibrio parahaemolyticus. Fish Shellfish Immunol Rep,2023:4:1-7.
5. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). Vet Res Forum,2014:5(2):95-100.
6. Chaves JO, de Souza MC, da Silva LC, Lachos-Perez D, Torres-Mayanga PC, Machado APDF, *et al*. Extraction of Flavonoids From Natural Sources Using Modern Techniques. Front Chem,2020:8:507887.
7. Dahija S, Cakar J, Vidic D, Maksimović M, Parić A. Total phenolic and flavonoid contents, antioxidant and antimicrobial activities of *Alnus glutinosa* (L.) *Gaertn.*, *Alnus incana* (L.) *Moench* and *Alnus viridis* (Chaix) DC. extracts. Nat Prod Res,2014:28(24):2317-2320.
8. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites,2012:2(2):303-336.
9. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. J Exp Pharmacol,2023:8(15):51-62.
10. Ernst O, Zor T. Linearization of the Bradford protein assay. J Vis Exp,2010:12(38):1918.
11. Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. Food Chem,2007:100(4):1511-1516.
12. Ighodaro OM, Akinloye OA. First line defence antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med,2018:54(4):287-293.
13. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (*In Vitro*). Maedica (Bucur),2019:14(4):350-356.
14. Kholieqoh AH, Kassim MNI, Muhammad TST, Anam K, Sung YY, Amir H, *et al*. SNEDDS to improve the bioactivities of *Pandanus tectorius* leaves: Optimization, antioxidant, and anticancer activities via apoptosis induction in human cervical cancer cell line. J Appl Pharm Sci,2024:14(10):175-189.
15. Kowalska T, Sajewicz M. Thin-Layer Chromatography (TLC) in the Screening of Botanicals-Its Versatile Potential and Selected Applications. Molecules,2022:27(19):6607.
16. Kumar NR, Sanjeeva PD. Antioxidant activity of methanol extract of Pandanus fascicularis Lam. J Pharm Res,2011:4(4):1234-6.
17. Lunga PK, Qin X-J, Yang XW, Kuiate JR, Du ZZ, Gatsing D. Antimicrobial steroidal saponin and oleanane-type triterpenoid saponins from Paullinia pinnata. BMC Complement Med Ther,2014:14:369.
18. Mohammed RS, Abou Zeid AH, El Hawary SS, Sleem AA, Ashour WA. Flavonoid constituents, cytotoxic and antioxidant activities of Gleditsia triacanthos L. leaves. Saudi J Biol Sci,2014:21:547-553.
19. Pant DR, Pant ND, Saru DB, Yadav UN, Khanal DP. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of Pterocarpus marsupium Roxburgh. J Intercult Ethnopharmacol,2017:6(2):170-176.
20. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, *et al*. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev, 2017, 8416763.
21. Raj GG, Varghese HS, Kotagiri S, Vrushabendra Swamy BM, Swamy A, Pathan RK. Anticancer Studies of Aqueous Extract of Roots and Leaves of Pandanus Odoratissimus f. ferreus (Y. Kimura) Hatus: An *In Vitro* Approach. J Tradit Complement Med,2014:4(4):279-284.
22. Ramani R, Chaturvedi V. Flow cytometry antifungal susceptibility testing of pathogenic yeasts other than Candida albicans and comparison with the NCCLS broth microdilution test. Antimicrob Agents Chemother,2000:44:2752-2758.
23. Valgas C, De Souza SM, Smânia EFA. Screening methods to determine antibacterial activity of natural products. Braz J Microbiol,2007:38:369-80.
24. Valverde M, Lozano-Salgado J, Fortini P, Rodriguez-Sastre MA, Rojas E, Dogliotti E. Hydrogen Peroxide-Induced DNA Damage and Repair through the Differentiation of Human Adipose-Derived Mesenchymal Stem Cells. Stem Cells Int,2018:10:1615497.
25. Yen GC, Duh PD. Antioxidative properties of methanolic extracts from peanut hulls. J Am Oil Chem Soc,1993:70(4):383-6.
26. Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q. The Applications and Mechanisms of Superoxide Dismutase in Medicine, Food, and Cosmetics. Antioxidants (Basel),2023:12(9):1675.