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RESEARCH ARTICLE

Phytochemical Composition and Multifunctional Bioactivities of Pandanus tectorius Leaf Extract: Antioxidant, Anti-Inflammatory, Antibacterial and Cytotoxic Potentials

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Abstract: Pandanus tectorius a medicinal plant traditionally used in Southeast Asia and the Pacific is a promising source of bioactive phytochemicals. The present study investigated the phytochemical composition, antioxidant, anti-inflammatory, antibacterial, and cytotoxic activities of the methanolic leaves extract of Pandanus tectorius. Phytochemical screening confirmed the presence of bioactive compounds including flavonoids, alkaloids, diterpenes, glycosides, steroid and terpenoid. Antioxidant activity was evaluated using multiple in vitro assays. The extract exhibited hydroxyl radical scavenging activity with an IC_{50} of 290.39 µg/mL, DPPH radical scavenging with an IC_{50} of 295.90 µg/mL, ABTS radical scavenging with an IC_{50} of 389.99 µg/mL, ferric reducing antioxidant power (FRAP) with an IC_{50} of 300.78 µg/mL, and nitric oxide scavenging with an IC_{50} of 300.80 µg/mL. Anti-inflammatory activity was confirmed through protein denaturation inhibition (10–80% inhibition across 100–500 µg/mL), cyclooxygenase inhibition (2.4–22.5% across 100–500 µg/mL), albumin denaturation inhibition with an IC_{50} of 447.21 µg/mL, and HRBC membrane stabilization with an IC_{50} of 353.53 µg/mL, outperforming the standard diclofenac (IC_{50} 674.83 µg/mL). Antibacterial assays demonstrated concentration dependent inhibition with the extract producing zones of inhibition of 17 mm against Bacillus subtilis and 12 mm against Escherichia coli. Furthermore, cytotoxicity evaluation against NCM460 human colonic epithelial cells revealed strong activity with an IC_{50} of 1.27 µg/mL. These findings highlight the therapeutic potential of P. tectorius leaves as a promising natural source of antioxidants, antiinflammatory agents and antibacterial compounds with relevance for managing oxidative stress, inflammatory disorders and microbial infections.

Keywords: Pandanus tectorius, Phytochemicals, Antioxidants, Antiinflammatory Antibacterial activity, Cytotoxicity.

INTRODUCTION

Inflammatory bowel diseases (IBDs) are a collection of inflammatory gut region disorders [1]. IBD usually ends with belly pain, gut muscle cramps, diarrhoea, and blood in stools [2]. Failure to cure it at the initial stage may lead to the tearing of the intestinal wall fistula [3]. The interaction of hereditary and environmental elements defines the immune reactions that cause IBDs. Mainly, inflammatory bowel illnesses are separated into Crohn's disease (CD) and ulcerative colitis (UC). Like UC, Crohn's disease has been classified as chronic IBD and it also creates digestive problems and inflammation in the gastrointestinal tract [2]. Usually, IBD causes weight loss, gastrointestinal pain, diarrhoea and rectal bleeding. Prolong conditions manifest with psychological imbalances [4] which needs proper treatment and diet [5]. The way IBDs work at a molecular level involves the creation of substances that cause inflammation and an overactive immune response when harmful microbes enter the body. The conventional treatment of IBDs involves reducing inflammatory conditions by neutralising the free radicals developed in the gut area. For example, traditional treatment of IBDs includes medications like corticosteroids, aminosalicylates, immunosuppressants, immunomodulators and biological agents such as antitumour necrosis factors, antiintegrins and antileukins [1,6]. The use of these drugs can disturb the gut microbiota [7,8] and affect vital organs [9]. But plant based products are usually safer. The IBD patients are increasingly using the products derived from plants and herbals. So, looking at the healing properties of certain plants can help reduce the need for synthetic drugs in treating IBD [10]. Pandanus tectorius (Pandanaceae) is otherwise known as screwpine. Ethnically, its aerial roots are used to treat blennorrhoea and its roots are helpful to control haemorrhoids. Its flower has aphrodisiac properties and the leaves control

colds/flu, hepatitis and boils [11]. Fruit of Pandanus tectorius is rich in significant phytochemicals such as phenol, flavonoid, steroid, terpenoid, alklaoid, saponin and glycosides [12]. Pandanus tectorius fruits and florals have antiinflammatory, antioxidant, antibacterial, and cytotoxicity activity[12,13]. Islam and Tahara [14] described the structure of dihydroflavonols, (2R,3S)-(+)-3,5-dihydroxy-4,7-dimethoxydihydroflavonol and (2R,3R)-(+)-4,5,7-trimethoxydihydroflavonol were obtained from Lannea coromandelica stem bark. Cheng et al.,[15] added an extract from P. tectorius leaves to the food of Cyprinus carpio to help the fish fight off diseases by lowering oxidative stress. Mundo et al.,[16] conducted a study on metabolic profiling and COX-2 expression in an in vivo model. But no study much revealed the phytochemicals of leaf portion of P.tectorius towards irritable bowel disease. Also, no comprehensive in vitro study has assessed the antioxidant, anti-inflammatory and antimicrobial properties of P.tectorius leaf extract. The study planned to select the leaf extract that demonstrates the antioxidant and antiinflammatory capacity of the leaf portion of the P. tectorius. In view of that, the present study aimed to identify the pharmacologically active chemicals in the Pandanus tectorius leaves that mitigate the symptoms of IBD.

MATERIALS AND METHODS

Collection and Extraction

Pandanus tectorius leaves were collected from the Tholkappia Poonga (Adyar Eco Park). (13.0192° N, 80.2647° E) (Fig.1a). The specimen was deposited in the G.S. Gill Research Institute, Velachery, Chennai. Using tap water, the collected leaves were cleaned two to three times followed by single wash with sterile water (Fig.1b). The washed Pandanus tectorius leaves were dried in cotton cloth. Then leaves were placed in a hot air oven for 4 days. After 4 days, the dried leaves were ground into a fine powder and then weighed. Fifty grams of the leaves powder were steeped in 250 ml of methanol and left to stand for 24 hours. The mixture was then filtered (Fig.1c) and then incubated at room temperature to allow evaporation [17].

2.2 Phytochemical Analysis

Phytochemical screening of the methanolic extract (LcBME) was carried out using standard qualitative assays. Flavonoids were detected by the lead acetate test, in which 2 mL of the methanolic extract solution was treated with 1–2 drops of 10% lead acetate solution. The formation of a yellowish precipitate was taken as evidence of the presence of flavonoids. Alkaloids were identified by Wagner's method, where 2 mL of LcBME mixed with 2–3 drops of Wagner's reagent produced a brown or reddish precipitate. Diterpenes were assessed using the copper acetate method, in which 2 mL of extract combined with 1 mL of aqueous copper acetate yielded an emerald-green colour. Glycosides were confirmed by both Liebermann and Salkowski's tests. In the Liebermann test, 2 mL of acetic acid and 2 mL of chloroform were added to the aqueous crude extract, cooled and treated with concentrated H_2SO_4, producing a green colour. In Salkowski's test, the addition of concentrated H_2SO_4 to the aqueous extract produced a reddish-brown hue. Steroids were detected by the Salkowski test in which 5 mL of the methanolic extract solution was mixed with 2 mL of chloroform followed by careful addition of 2 mL of concentrated H_2SO_4 along the side of the test tube. The development of a red colour in the lower chloroform layer was taken as a positive indication of steroids. Terpenoids were identified by the Salkowski test, in which 5 mL of the methanolic extract solution was mixed with 2 mL of chloroform. The mixture was evaporated to dryness in a water bath and the residue was heated with 3 mL of concentrated H_2SO_4. The development of a greyish colour was considered a positive indication of terpenoids [18].

2.3 Antioxidant Analysis

2.3.1 Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of the extract was evaluated following the method of Harsha Ramakrishna et al. [19] using ascorbic acid as the reference standard and measuring absorbance at 532 nm. The percentage inhibition was calculated as: % Inhibition = ((Absorbance of control – Absorbance of sample) / Absorbance of control) × 100. A standard curve was constructed by plotting concentration against percentage inhibition and the IC_{50} value was determined from the regression equation. All assays were carried out in triplicate [20].

2.3.2 DPPH Radical Scavenging Assay

The antioxidant activity of the methanolic extract of Pandanus tectorius leaves was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with slight modifications of previously reported methods. The extract was dissolved in methanol to obtain solutions ranging from 100 to 500 µg/mL. For the assay, 1 mL of extract solution at different concentrations was mixed with 1 mL of 0.1 mM DPPH solution, vortexed and incubated in the dark at 25 ± 2 °C for 30 minutes. Butylated hydroxytoluene (BHT) was used as the positive control. Absorbance was measured at 517 nm against methanol as the blank using a UV–Vis spectrophotometer. The percentage of radical scavenging activity was calculated using the equation: % Inhibition = [(A control – A_sample) / A_control] × 100 where A_control is the absorbance of the control reaction and A_sample is the absorbance of the extract. The IC_{50} value defined as the concentration required to inhibit 50% of the DPPH radicals, was determined from dose–response curves using nonlinear regression analysis [21].

2.3.3 ABTS Radical Scavenging Assay

The ABTS radical scavenging activity of the extract was determined according to the standard protocol [22]. 100 µL of the sample at concentrations ranging from 100 to 500 µg/mL was mixed with 0.9 mL of ABTS solution which was prepared using 7 mM ABTS and 2.45 mM potassium persulfate in ethanol. The reaction mixture

was incubated in the dark at room temperature for 4–16 hours and the resulting solution was diluted with ethanol to obtain an absorbance of 0.706 ± 0.001 at 734 nm. After 15 minutes of reaction the absorbance was measured spectrophotometrically at 734 nm. The ABTS scavenging activity of the extract was compared with that of BHT and the percentage inhibition was calculated as: ABTS radical scavenging activity (%) = (OD control – OD test / OD control) × 100.

2.3.4 Ferric Reducing Antioxidant Activity

The ferric reducing antioxidant power (FRAP) assay was performed according to the standard protocol [23]. Sample aliquots at concentrations ranging from 100 to 500 µg/mL were diluted with distilled water to obtain a final volume of 1 mL which was then mixed with 3 mL of freshly prepared FRAP reagent and incubated at 37 °C for 30 minutes. After incubation, absorbance was measured at 593 nm against a reagent blank containing the complete reaction mixture except that the extract or standard was replaced with distilled water. A calibration curve was constructed using different concentrations of ferrous sulphate treated under identical conditions.

2.3.5 Nitric Oxide Scavenging Assay

Nitric oxide scavenging activity was determined according to the standard protocol. A 100 µL sample at concentrations ranging from 100 to 500 µg/mL was mixed with 1 mL of sodium nitroprusside (5 mM) in phosphate buffered saline (0.025 M, pH 7.4) and incubated at 29 °C for 3 hours. After incubation, 1 mL of the reaction mixture was combined with 1 mL of Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl) ethylenediamine and 2% orthophosphoric acid) in phosphate buffer (pH 7.4). The absorbance was measured spectrophotometrically at 550 nm. Ascorbic acid was used as the reference standard under identical conditions. The percentage inhibition was calculated as: % inhibition = ((OD control – OD test) / OD control) × 100 [24].

2.4 Anti-inflammatory Analysis

2.4.1 Inhibition of protein denaturation

Samples at different concentrations ranging from 100 to 500 µg/mL were prepared up to a final volume of 1 mL using methanol. To each tube, 5 mL of 0.2% bovine serum albumin (BSA) in Tris-buffered saline (pH 6.8) was added. In the control tube, 50 µL of methanol and 5 mL of 0.2% BSA were used. The tubes were incubated at 37 °C for 20 minutes followed by heating at 72 °C for 5 minutes and then cooled for 10 minutes. Absorbance was measured at 660 nm against Tris buffered saline as the blank. Diclofenac (1 mg/mL) was used as the standard. The percentage inhibition of protein denaturation was calculated as: % inhibition = [(A control – A sample) / A control] × 100 [25].

2.4.2 Cyclooxygenase inhibition assay

Test tubes contained different sample concentrations ranging from 100 to 500 µg/mL adjusted to a final volume of 1 mL using 0.1 N Tris-HCl buffer. To each tube, 100 µL of hemoglobin, glutathione and enzyme were added followed by 200 µL of arachidonic acid and trichloroacetic acid (TCA). The mixture was incubated at 37 °C for 20 minutes. After incubation, 200 µL of thiobarbituric acid (TBA) was added and the tubes were boiled in water for 20 minutes. The samples were then cooled, centrifuged at 1000 rpm for 3 minutes and the COX activity was determined at 632 nm using the supernatant. The percentage inhibition of COX activity was calculated using the formula: % inhibition = [(A control – A sample) / A control] × 100 [26].

2.4.3 Inhibition of Albumin Denaturation

Test tubes were prepared with varying sample concentrations ranging from 100 to 500 µg/mL and the volume was adjusted to 1 mL using phosphate buffer saline (pH 6.4). To each tube, 0.5 mL of a 5% aqueous solution of bovine serum albumin was added. The pH of the mixture was adjusted to 6.3 with 0.1 N HCl thoroughly mixed and incubated at 37 °C for 20 minutes followed by heating at 57 °C for 3 minutes. After cooling the absorbance (A) of both the test samples and the control was measured at 660 nm. Diclofenac (1 mg/mL) served as the standard while the control was prepared without the sample. The percentage inhibition of albumin denaturation was calculated using the formula: % inhibition = [(A control – A sample) / A control] × 100 [27].

2.4.4 Membrane Stabilizing Property

Freshly collected blood (1 mL) was centrifuged at 3,000 rpm for 5 min and mixed with an equal volume of Alsever's solution containing 2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl. The red blood cells (RBCs) were isolated by discarding the supernatant, washed with isosaline (0.85% NaCl) at 3,000 rpm for 5 min and resuspended by diluting 1 mL RBCs in 9 mL isosaline to obtain the HRBC suspension. Different sample concentrations ranging from 100 to 500 µg/mL were prepared in 1 mL distilled water, to which 1 mL phosphate buffer, 1 mL hypotonic saline (0.36% NaCl) and 1 mL HRBC suspension were added. The mixtures were incubated at 37 °C for 30 min and centrifuged at 3,000 rpm for 20 min. Absorbance of the supernatant was recorded at 560 nm. Diclofenac (1 mg/mL) was used as the standard and the control was prepared without the sample. The percentage of HRBC membrane protection was determined using the equation: Percentage protection = 100 – (OD sample / OD control × 100) [25].

2.5 Antibacterial Activity by Agar well diffusion method

Bacterial stock cultures were maintained on nutrient agar slants at 4 °C. For experimental use, a loopful of culture was inoculated into nutrient broth and incubated at 37 °C for 24 h to obtain actively growing bacterial suspensions. The antibacterial activity of the plant extract was evaluated using the agar well diffusion method on Mueller Hinton agar (MHA) medium. The medium was

sterilized, poured into sterile Petri plates, and allowed to solidify. Sterile cotton swabs were dipped into the bacterial suspension and evenly spread over the agar surface. Wells were then made aseptically using a sterile cork borer. Each well was loaded with the test sample at different concentrations ranging from 20 to 80 µg/mL while streptomycin (100 µg /mL) served as the positive control. Plates were incubated at 37 °C for 24 h. Antibacterial activity was assessed by measuring the diameter of the zone of inhibition around each well in millimeters (mm)[28].

2.6 Cytotoxicity Analysis (MTT assay)

NCM460 cells were subcultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS). After discarding the culture medium, cells were trypsinized and resuspended in 25 mL of fresh DMEM by gentle pipetting. A 24-well plate was seeded with 1 mL of the homogenized cell suspension per well along with varying concentrations of the plant extract ranging from 0 to 800 µg/mL. The plates were incubated at 37 °C in a humidified CO_2 incubator with 5% CO_2 for 48 h until cells reached approximately 80% confluence. Following incubation, the medium was removed and 100 µL of MTT solution was added to each well. The plates were kept at room temperature for 3 h to allow mitochondrial succinate dehydrogenase of viable cells to reduce MTT into insoluble purple formazan crystals. The supernatant was then removed and 100 µL of SDS in DMSO was added to dissolve the crystals. Absorbance was measured at 540 nm using a microplate reader (Lark LIPR-9608). Cytotoxicity was expressed as: Cytotoxicity (%) = 100 – (OD sample / OD control × 100). The IC_{50} value defined as the concentration of the extract required to inhibit 50% of cell viability was determined from the dose response curve [29].

RESULTS AND DISCUSSION

3.1 Extraction and determination of phytochemicals

The results confirmed the presence of diverse phytochemicals in the methanolic extract of Pandanus tectorius leaves as summarized in Table 1 and illustrated in Fig 2.

Table 1: Phytochemicals screening of Pandanus tectorius leaf's methanolic extract

Phytochemicals	Results
Flavonoids	+
Alkaloid	+
Diterpenes	+
Glycoside (Libermann test)	+
Glycoside (Salkowski test)	+
Steroid	+
Terpenoid	+

Table 2. Antibacterial activity of Methanolic extract of Pandanus tectorius leaves

Microorganisms	Zone of Inhibition in mm				
	1	2	3	4	5
Bacillus subtilis	11	13	15	17	22
Escherichia coli	-	-	11	12	20

1-20 µg/mL 2- 40 µg/mL 3- 60 µg/mL 4- 80 µg/mL 5- Streptomycin 100 µg/mL

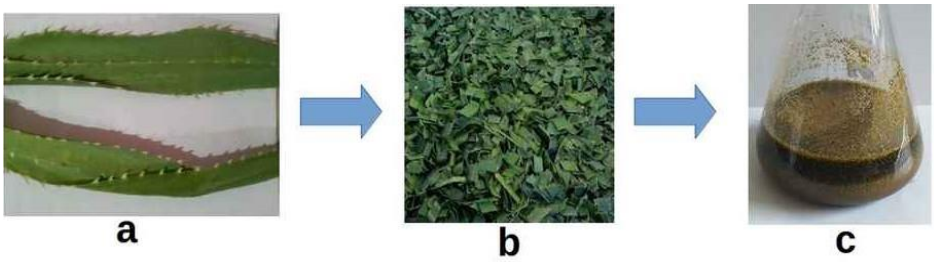


Figure 1. Collection of Pandanus tectorius leaves and extraction

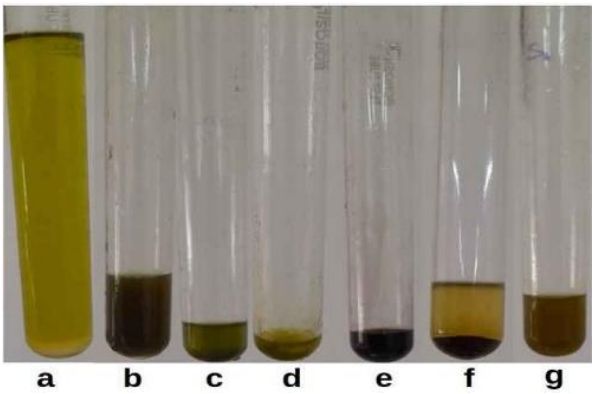
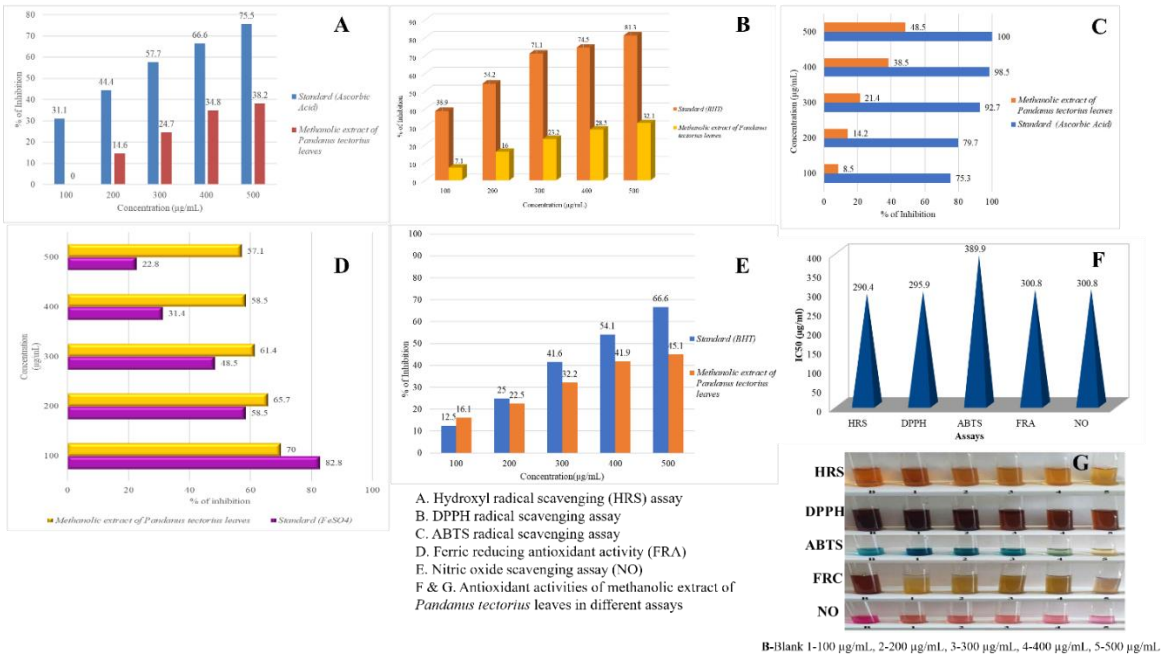
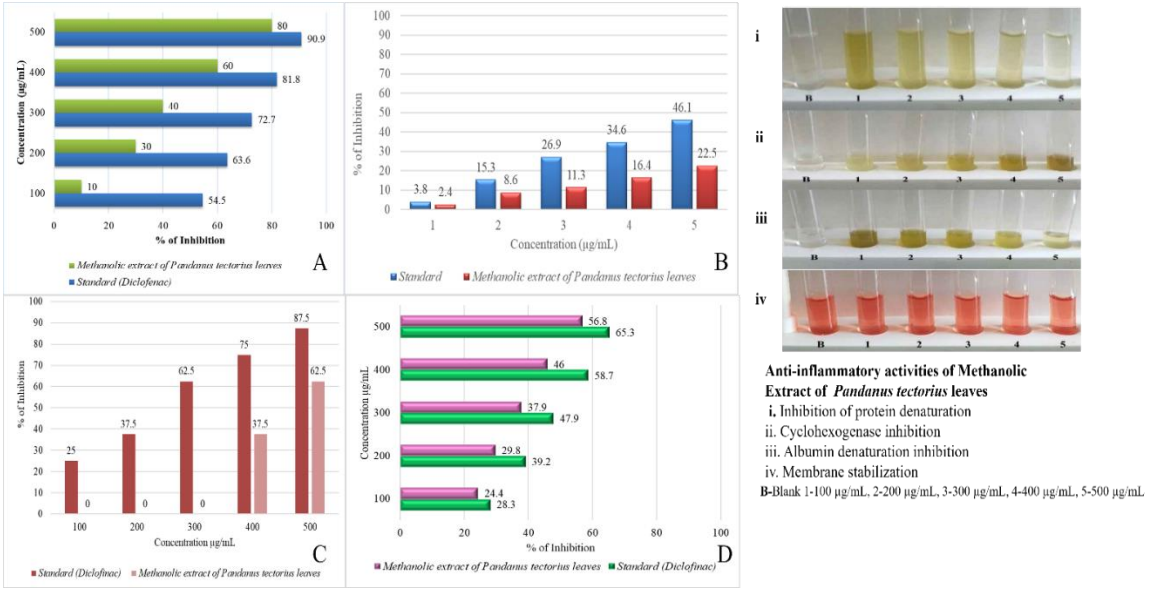


Figure 2. Phytochemicals of methanolic extract of Pandanus tectorius leaves (a. Flavonoids, b. Alkaloid, c. Diterpenes, d&e. Glycoside, f. Steroid, g. Terpenoid)



A. Hydroxyl radical scavenging (HRS) assay
B. DPPH radical scavenging assay
C. ABTS radical scavenging assay
D. Ferric reducing antioxidant activity (FRA)
E. Nitric oxide scavenging assay (NO)
F & G. Antioxidant activities of methanolic extract of *Pandanus tectorius* leaves in different assays

B-Blank 1-100 µg/mL, 2-200 µg/mL, 3-300 µg/mL, 4-400 µg/mL, 5-500 µg/mL



Anti-inflammatory activities of Methanolic Extract of *Pandanus tectorius* leaves
i. Inhibition of protein denaturation
ii. Cyclohexogenase inhibition
iii. Albumin denaturation inhibition
iv. Membrane stabilization
B-Blank 1-100 µg/mL, 2-200 µg/mL, 3-300 µg/mL, 4-400 µg/mL, 5-500 µg/mL

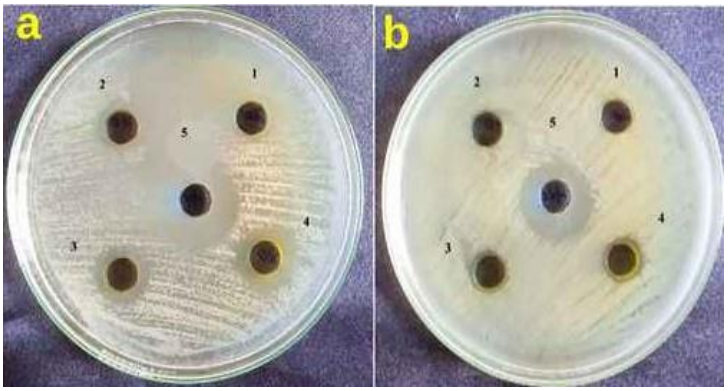


Figure 5. Antibacterial activities of Pandanus tectorius leaf extract a. Bacillus subtilis b. E.coli 1-20 µg/mL 2- 40 µg/mL 3- 60 µg/mL 4- 80 µg/mL 5- Streptomycin 100 µg/mL

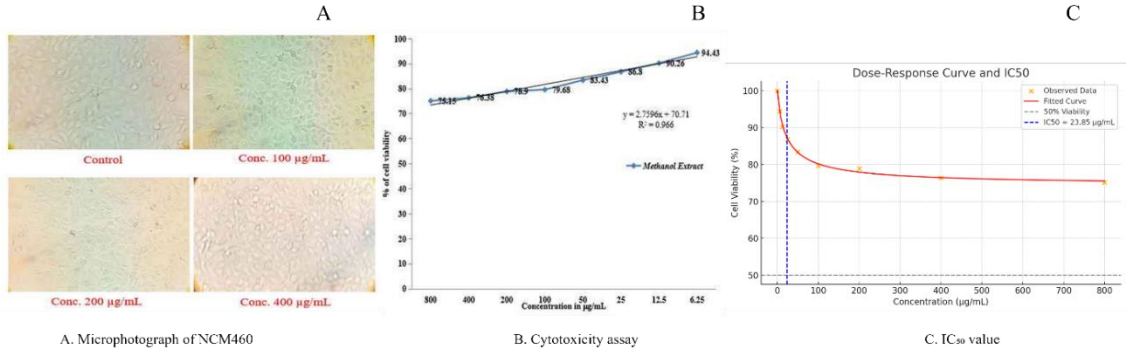


Figure 6. Cytotoxicity and IC_{50} of methanolic extract of Pandanus tectorius leaves against human colonic epithelial cells NCM460

3.2 Antioxidant activity

3.2.1 Hydroxyl radical activity

The hydroxyl radical scavenging (HRS) assay was employed to assess the antioxidant potential of the methanolic extract based on its ability to neutralize hydroxyl radicals highly reactive species implicated in oxidative damage. The assay was performed using the deoxyribose degradation method where inhibition of hydroxyl radical mediated deoxyribose damage is indicated by a straw-yellow colour (Fig 3). The extract exhibited a concentration dependent scavenging effect achieving maximum activity at 500 µg/mL with an IC_{50} value of 290.40 µg/mL. Previous studies have demonstrated that plant extracts exert antioxidant activity by stimulating the expression of key antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD1) and glutathione peroxidase (GPx1) as well as phase II detoxifying enzymes such as haemeoxygenase-1 (HO-1) through upregulation of the nuclear factor erythroid 2–related factor 2 (Nrf2) mediated pathway in monocyte/macrophage like RAW 264.7 cells [30,31]. The strong radical scavenging activity of the extract highlights its potential to mitigate oxidative stress by neutralizing hydroxyl radicals among the most deleterious reactive oxygen species thereby supporting its therapeutic relevance in oxidative stress associated disorders such as inflammatory bowel disease (IBD).

3.2.2 DPPH assay

The antioxidant potential of the methanolic extract was further evaluated using the DPPH radical scavenging assay a well-established method for assessing lipid-soluble antioxidants. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical characterized by a deep violet colour, which becomes pale yellow upon reduction by antioxidants (Fig. 3). The extract demonstrated a dose dependent scavenging effect with %RSA increasing from 7.1% at 100 µg/mL to 32.1% at 500 µg/mL. The calculated IC_{50} value was 295.90 µg/mL. Comparable findings were reported by Andriani et al. [12], who studied the fruit extract of P. tectorius and observed an IC_{50} value of 2 mg/mL. The stronger scavenging potential observed in the present study emphasizes the importance of the leaf extract in antioxidant activity. Similarly, Tekeshwar and Vishal [32] reported that crude methanolic and acetate extracts of L. coromandelica leaves demonstrated significant radical scavenging capacity supporting the efficacy of methanolic leaf extracts in antioxidant assays.

In vivo studies further validate these findings, as P. tectorius leaf extract was shown to enhance levels of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) while reducing malondialdehyde (MDA) in the liver and kidney of CCl_4-intoxicated rats [33]. Lipid peroxidation (LP) represents a key manifestation of oxidative radical mediated injury closely associated with ferroptosis and secondary damage to essential biomolecules such

as proteins. The ability of the extract to attenuate LP therefore underscores its therapeutic potential in mitigating oxidative stress driven pathologies. The significant DPPH radical scavenging activity of P. tectorius leaf extract highlights its potential as a natural antioxidant. These findings support its application in mitigating oxidative stress associated conditions particularly inflammatory bowel disease (IBD) where oxidative damage plays a pivotal role in disease pathogenesis.

3.2.3 ABTS assay

The antioxidant potential of the extract was further assessed using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay (Fig 3). The extract demonstrated complete inhibition (100%) at 500 µg/mL with an IC_{50} value of 389.99 µg/mL indicating a dose dependent activity. These findings are consistent with earlier reports wherein potent ABTS radical inhibition by Cyperus rotundus and Acacia nilotica extracts was demonstrated by Rajurkar et al. [34]. Similarly, Shahinuzzaman et al. [35] reported that Ficus carica latex exhibited notable ABTS scavenging activity (27.08 ± 0.34 mg TE/g latex). Collectively, the present results highlight the robust free radical quenching capacity of P. tectorius leaf extract further substantiating its potential as a natural antioxidant source.

3.2.4 Ferric reducing antioxidant activity

The ferric reducing antioxidant power (FRAP) assay revealed that the methanolic leaf extract of Pandanus tectorius possessed notable electron donating ability, reducing Fe^{3+} to Fe^{2+} as indicated by the characteristic blue colouration measured at 593 nm (Fig. 3). The extract showed 82.8% reducing activity at 100 µg/ml decreasing to 22.8% at 500 µg/ml with an IC_{50} of 300.78 µg/ml confirming a moderate yet significant antioxidant potential. These findings are in agreement with the report of Seleshe et al. [36] who demonstrated extended radicals scavenging activity of flavonoid rich herbal plants using FRAP analysis. Phytochemical screening in the current study also confirmed the presence of flavonoids thereby supporting their role as key contributors to the reducing capacity of the extract. Similarly, Andriani et al. [12] identified five flavonoid compounds from the fruit of P. tectorius where the total antioxidant capacity was measured with an IC_{50} of 0.8 ± 0.20 mg/ml. Comparative analysis highlights that the methanolic leaves extract exhibits greater antioxidant potential than the fruit extract further validating the therapeutic significance of the leaves as a potent natural antioxidant source.

3.2.5 Nitric oxide scavenging activity

Nitric oxide (NO) is a free radical involved in normal metabolism, but its excessive production is linked to chronic inflammation such as IBD. In this study, the methanolic leaves extract of P. tectorius showed dose dependent NO scavenging activity (Fig. 3). At 100 µg/ml, the extract showed 16.1% inhibition which increased to 45.1% at 500 µg/ml confirming a dose dependent effect. The IC_{50} value was calculated as 300.8 µg/ml. Comparable findings were reported by Patel et al. [37] who demonstrated nitric oxide scavenging activity in Tephrosia purpurea leaves extract, and by Boora et al. [38] who observed nitrite radical scavenging activity in aqueous and ethanolic leaves extracts of C. zeyheri, C. platypetalum, and P. curatellifolia.

Fig 3 further presents a comparative analysis of IC_{50} values across different antioxidant assays. Among these, the hydroxyl radical scavenging (HRS) assay exhibited the strongest inhibitory effect followed by DPPH, FRAP, NO, and ABTS. Hydroxyl radicals being highly reactive generate reactive oxygen species (ROS) that cause oxidative damage to biomolecules including proteins and DNA. Phytochemical analysis confirmed the presence of flavonoids in P. tectorius leaves extract which likely contribute to the neutralization of such reactive radicals thereby reducing risks associated with oxidative stress related disorders such as cardiovascular disease and cancer [39].

3.3 Anti-inflammatory activity

3.3.1 Inhibition of Protein denaturation

Proteins are fundamental to biological function and their structural integrity is often compromised during inflammation leading to loss of activity. Preventing protein denaturation is therefore critical in mitigating inflammatory damage. In the present study, the methanolic leaf extract of P. tectorius was evaluated for its ability to inhibit protein denaturation (Fig 4). The extract demonstrated a clear dose dependent effect with inhibition increasing from 10% at 100 µg/ml to 80% at 500 µg/ml. Comparable findings were reported for Justicia secunda Vahl leaf extract which exhibited 83.08% and 89.05% inhibition at 100 and 300 mg/ml respectively, in carrageenan and formaldehyde induced in vivo models [40]. Collectively, these results highlighted the significant antiinflammatory potential of P. tectorius through its capacity to stabilize protein structure under stress conditions.

3.3.2 Cyclooxygenase inhibition test

Cyclooxygenase (COX) catalyzes the oxidation of arachidonic acid to cycloendoperoxides the precursors of key pro-inflammatory mediators including prostaglandins, leukotrienes and thromboxanes. Although physiologically expressed in cells elevated COX activity is strongly associated with chronic inflammation and carcinogenesis making it a critical therapeutic target for nonsteroidal antiinflammatory drugs (NSAIDs). In the present study, the methanolic leaves extract of P. tectorius exhibited a dose dependent inhibitory effect on COX activity (Fig 4) with inhibition increasing from 2.4% at 100 µg/ml to 22.5% at 500 µg/ml. Standard drugs such as diclofenac and meloxicam displayed balanced inhibition of COX isoenzymes [41]. Similarly, extracts from Acacia seyal, Capparis decidua, Combretum hartmannianum and Ziziphus spina-christi

have been reported to effectively inhibit COX activity [42]. These findings support the potential of P. tectorius as a natural antiinflammatory agent though with lower potency compared to standard NSAIDs.

3.3.3 Albumin denaturation inhibition activity

Albumin denaturation results from the disruption of weak hydrogen bonds and hydrophobic interactions that stabilize its globular structure and serves as a key marker of inflammatory conditions such as inflammatory bowel disease (IBD). In the present study, the methanolic leaf extract of P. tectorius demonstrated inhibition of albumin denaturation in a dose dependent manner (Fig 4) with 37.5% inhibition observed at 400 µg/ml. The IC_{50} value was calculated as 447.21 µg/ml indicating moderate but effective protective activity. This effect is likely attributable to the flavonoid constituents of the extract. The findings are in strong agreement with those of Okeke et al. [43] who reported flavonoid rich plant extracts to inhibit albumin denaturation and with Dharmadeva et al. [44] who demonstrated similar protective effects using Ficus racemosa bark extract.

3.3.4 Membrane stabilizing property

The stabilization of cellular membranes is critical for protecting cells under stress and preventing hemolysis of red blood cells. In the present study, the methanolic leaves extract of P. tectorius exhibited notable membrane stabilizing activity with an IC_{50} value of 353.53 µg/ml, outperforming the standard drug diclofenac (674.83 µg/ml) (Fig. 4). These results highlighted the strong protective potential of the extract. Comparable findings have been reported in other medicinal plants showed significant membrane stabilization with extracts of Arctium tomentosum, Gnaphalium kasachstanicum and Artemisia vulgaris [45] while Sonter et al. [46] confirmed similar effects with Murraya paniculata. Likewise, Anosike et al. [47] showed that Mucuna pruriens extracts effectively stabilized membranes in both healthy and sickle cell erythrocytes.

3.5 Antibacterial activity

The antibacterial potential of the methanolic extract of Pandanus tectorius leaves was assessed against Bacillus subtilis and Escherichia coli using the agar well diffusion method, with results summarized in Table 2. The extract exhibited a clear concentration-dependent inhibitory effect. Against B. subtilis, zones of inhibition increased from 11 mm at 20 µg/mL to 17 mm at 80 µg/mL whereas the reference antibiotic streptomycin (100 µg/mL) produced a maximum inhibition of 22 mm. In contrast, no inhibition of E. coli was detected at 20 or 40 µg/mL. However, activity became evident at higher concentrations producing inhibition zones of 11 mm and 12 mm at 60 and 80 µg/mL, respectively, compared with 20 mm for streptomycin. These results indicated that the extract is more effective against the Gram-positive strain (B. subtilis) than the Gram-negative strain (E. coli), consistent with the inherent resistance of Gram-negative bacteria attributed to their outer membrane barrier. Similar findings have been reported in earlier studies. Anirudhan et al. [48] demonstrated that P. tectorius leaf extract suppressed Vibrio parahaemolyticus infections in white leg shrimp (Penaeus vannamei). Likewise, Andriani et al. [49] confirmed the antibacterial activity of P. tectorius fruit extract against B. subtilis, E. coli and Klebsiella pneumoniae.

3.6 Cytotoxic activity

NCM460 refers to normal human colonic epithelial cells that are widely used in research as an in vitro model for studying gastrointestinal physiology, pathology, and pharmacology. These cells are particularly relevant in inflammatory bowel disease (IBD) research, as they provide insights into epithelial barrier function, inflammation, and cellular responses to various stimuli or therapeutic agents. In the current study, the extract has produced IC50 value of 1.2768µg/ml (Figure 6). The viability of the NCM460 cells is dose dependent. Ding and Wen[50] proved that dandelion root extract reduced the ROS species in NCM460 cells by blocking the nuclear factor-kappaB signalling. NF-kB, a transcription factor, regulates inflammatory activities. Dysregulated NF-kB activity leads to inflammation-related diseases including cancers[51]. Part of the signal transduction pathway including protease-dependent receptor signalling, NF-kB is absolutely essential for controlling inflammation and immunological activation[52]. Schneider et al.,[53] tested herbal extracts inflammatory activities in cytokine challenged NCM460 cells. The study proved that the extracts were influencing the cytokine production. Blocking NF-kB signaling is helpful treat the inflammatory disorders such as IBD, rheumatoid arthritis, multiple sclerosis, and atherosclerosis [52]. The anti- inflammatory study results and cytotoxic studies were highly correlated in this study. The methanolic extract of Excoecaria agallocha exhibited strong in vitro anticancer effects against human cervical (HeLa) and breast (MDA-MB-231) cancer cell lines, showing IC50 values of 19.50± 0.41µg/mL and 20.67± 0.14 µg/mL, respectively [53].

CONCLUSION

The research on Pandanus tectorius leaf extract reveals promising potential as a natural, non- toxic therapeutic option for treating inflammatory bowel disease, highlighting its significant antiinflammatory and antioxidant properties while paving the way for future clinical trials. Secondary metabolites have demonstrated significant tumour reduction in cancer cell lines and mouse models. Investigating natural products remains a promising approach for discovering and developing new bioactive compounds with unique modes of action[54,55]. This study not only supports traditional uses of the plant but also opens new avenues for safer alternative therapies in managing chronic inflammatory disorders.

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Ethics Statement
This study did not involve human participants, animal subjects, or the use of any material requiring ethical approval.

Informed Consent Statement
This research did not involve human participants; hence, informed consent was not applicable.

Clinical Trial Registration
This study does not involve any clinical trials and therefore does not require registration.

Permission To Reproduce Material From Other Sources
Not applicable.

Author Contributions
Ramesh.T contributed to the conceptualization, experimental work and data curation. A.Amuthavalli was responsible for supervision, methodology development and review and editing. P. Sankarganesh handled validation, formal analysis and data visualization. Ganesh Kumar A conducted microbiological assays and provided laboratory support. R. Emimal was involved in plant material collection and phytochemical screening. Manivannan. G provided overall supervision, managed the project administration and prepared the final draft of the manuscript.

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