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In Vitro Assessment of Antioxidant, Thrombolytic, Antimicrobial Activities of Medicinal Plant *Pandanus odoratissimus* L. Leaves Extract

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Abstract

The present study was carried out to investigate phytochemical, antioxidant; antimicrobial, thrombolytic activity and estimate total phenolic, total flavonoid content of *Pandanus odoratissimus* (*P. odoratissimus*) leaves of methanol extract. In thrombolytic activity, aqueous soluble fraction (AQSF) exhibited highest percentage (46.58 %) of potential to lyse blood clot compared to standard drug streptokinase (69.52 %). In antimicrobial assay, dichloromethane soluble fraction (DCMSF) explored the highest diameter of clear zone of inhibition against both gram positive (19.60 ± 0.12 mm) and gram negative (20.00 ± 0.20 mm) bacteria compared to standard antibiotic, Kanamycin (50.00 ± 0.19). Levels of antioxidant were determined by DPPH assay followed by calculated IC_{50} values of different Kupchan extracts. The methyl soluble fraction (MSF) showed the lowest level of IC_{50} value (36.70 ± 0.32 µg/mL) in comparison to ascorbic acid (12.48 ± 0.09 µg/mL) while MSF disclosed the maximum level (62.19 ± 0.26 mg of GAE/g of extract) of total phenolic content in the extracts of *P. odoratissimus.* This study was conducted to validate the *P. odoratissimus* leaves used as a folk medicine such as, antioxidant, thrombolytic, and antimicrobial potential.

Keywords: *Pandanus odoratissimus*; Thrombolytic; Antimicrobial; Antioxidant activity.

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1. Introduction

Plants are the ample source of bioactive substances that have been used traditionally since human civilization [1]. Major bioactive compounds such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins, and polyphenols are responsible for the pharmacological activity [2]. The antimicrobial compounds from leaves of *Pandanus odoratissimus* may inhibit bacterial propagation by different mechanisms

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than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains [3]. Thrombosis is the mechanism by which formation of a blood clot, known as a thrombus, happens within a blood vessel. It prevents blood from flowing normally through the circulatory system therefore depriving tissues of normal blood flow and oxygen. These consequences yield necrosis of the tissue in that area leading hypertension, stroke to the heart, anoxia, and so on. Thrombo embolic disorders are one of the main causes of morbidity and mortality in Bangladesh [4]. Oxidative stress is closely related with the pathogenesis of life-style diseases such as atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies [5]. Oxygen consumption during metabolism generate different kinds of oxygen free radicals (ROS) namely superoxide radical, hydrogen peroxide, hydroxyl radical, singlet oxygen or nitrogenous substances (RNS) that can be hazardous for biological systems [6]. Majority of the ROS or RNS are removed or neutralized by the presence of enzymatic or non-enzymatic processes [7]. Excess ROS can cause tissue damage which is thought to be the root cause of several other diseases such as arthritis, vasculitis, nephritis, emphysema, lupus erythematous, Alzheimer's diseases, Parkinson's diseases etc. [8]. *Pandanus odoratissimus* L. (Syn. *P. fascicularis*) belonging to family *Pandanaceae*, is widely distributed along Indo-Malayan coasts of India and Sri-Lanka, throughout Southeast Asia to Taiwan, the Ryukyu Islands, Malaysian islands and Australia. The plant is commonly known as 'Kevda' in Hindi, 'Umbrella tree' in English, and 'Kaethakee' in Sanskrit, Ketaki in Bangla. *P. odoratissimus* is a dioecious shrub, densely branched with copious aerial roots. Leaves are caudate acuminate, glaucous green, 90–150 cm long, curvaceous margin with ascending spinules (toothed) spadices axillaries, terminal, simple, branched, clothed with leafy spathes. Flowers are small crowded on a catkin like spadix and its branches [9]. The leaves contain the pyridine alkaloids, pandamarilactone-1($C_{18}H_{23}NO_4$), pandamarilactone-31($C_{19}H_{25}NO_4$), pandamarilactone-32($C_{18}H_{21}NO_3$). The aroma compound 2-acetyl-1-pyrollidine has been identified from the volatile oil of the leaf [10]. Lignans and benzofurans have been isolated from roots of *Pandanus odoratissimus* [11]. This study shows that the methanol extract of leaves of *P. odoratissimus* has bioactivity but further compound isolation is necessary to confirm the activities of individual compounds.

2. Materials and Methods

2.1. *Drugs and Chemicals*

Streptokinase, SK (1.5 million unit/vial, streptase®) vial was warmly gifted from Sanofi-Aventis Bangladesh Ltd. Gallic acid (GA), sodium carbonate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), kanamycin was obtained as gift from Amico laboratories limited, Bangladesh. Different solvents such as methanol, petroleum ether, dichloromethane, chloroform and other necessary chemicals and reagents were

of highest analytical grade and collected from the Bashar Chemicals and Equipment, Dhaka.

2.2. Plant assortment, detection and authentication, drying and grinding

The whole plant of *P. odoratissimus* was collected from Botanical Garden, Mirpur, Dhaka and identified by taxonomist of National Herbarium, Mirpur, Dhaka, Bangladesh. The Voucher Specimen No. is ICAB: 66523. The sample is preserved in the Phytochemical Research Laboratory, World University of Bangladesh. The collected plant of *P. odoratissimus* was cleaned properly to remove unwanted debris and air dried in shady place for several days. The dried plants were then milled by locally made grinder in order to get uniform coarse powder. The coarse powder (300 g) was further milled and stockpiled in a hermetic glass container till extraction.

2.3. Extraction and fractionation

The powdered material (250 g) was taken in a cleaned, amber colored reagent bottle (5 L) and soaked in 2.5 L (1:2.5 w/v) of 90 % methanol for 2 weeks at room temperature (25±1 ºC). The dark brown color decoction was filtered after 2 weeks. The filtration output was desiccated applying the rotary evaporator (IKA® RV 10 Basic, India) at 40 ºC, low pressure and 125-175 rpm. The retrieved solvent was reused for next consecutive refluxes carried out after 24 h of additional soaking. The crude methanol extract (52 g) was applied to fractionation by Kupchan protocol [12] and modified by Wagenen *et al.* [13]. The crude extract was dissolved in 10 % aqueous methanol and subsequently extracted with petroleum ether, dichloromethane, and finally with chloroform. The fractionated amounts were petroleum ether soluble fraction (PSF= 16.5 g), dichloromethane soluble fraction (DCMSF = 12.5 g) and aqueous soluble fraction (AQSF = 8 g), respectively. All the organic fractions were applied to evaporation to dryness and used for further analysis.

2.4. *Phytochemical screenings*

Qualitative experimentation on different Kupchan partitionates was conducted using the standard concordat developed by Sofowara [14]. The method supported to unveil the qualitative sustenance of phytochemicals such as alkaloids, flavonoids, steroids, phenols, resins, glycosides, and saponins in the crude extracts and its numerous organic soluble fractions.

2.5. *Preparation of streptokinase solution*

The commercially available lyophilized SK was used as positive control. The lyophilized vial was collected and whole powder was dissolved in 100 mL water to get

1,500,000 I.U. of SK solution. This suspension was used as a stock from which 100 µL (30,000 I.U) was used for the determination of *in vitro* thrombolytic potentials.

2.6. *Estimation of total phenolic content (TPC)*

In the alkaline condition phenols from plant extracts ionize completely. The TPC was determined by using Folin-Ciocalteu [15] reagent. The test was executed by attributing the proposed method with slight modification [16]. 2 mg crude methanolic extract and its different organic soluble fractions were liquefied with 2 mL distilled water to evoke the final concentration at 1 mg/mL. 0.5 mL of extractives (1 mg/mL) was combined with 2 mL Folin-Ciocalteu (diluted 10-fold with deionized water previously) in the test tube. The concoction was allowed to stand for 5 min at 22 ± 2 ºC. Each mixture was then added to 2.5 mL 7.5 % Na_2CO_3. The combination was gently shaken and allowed for no major jerking for 20 min for color development. The intensity of the color change was measured by UV-Vis spectrophotometer (Model: UV-1700 series) method at 760 nm. The absorbance value reflected TPC of the compound. Samples of extracts and standard were appraised at a final concentration of 0.1 mg/mL. TPCs were expressed in terms of GA equivalent, GAE (Standard curve equation: $y = 0.0162x + 0.0215$, $R^2 = 0.9985$), mg of GA/g of dry extract [17].

2.7. *Determination of total flavonoid content*

The total flavonoid content of crude methanolic extract its partitionates (PSF, DCMSF, AQSF) were determined by the aluminum chloride colorimetric method [18]. In brief, 1.5 mL of crude extract (1 mg/mL methanol) was mixed with 0.1 mL of 10% $AlCl_3$ and then 0.1 mL of 1 M Na-acetate was added to the reaction mixture. The mixture was allowed to stand for 6 min. Then, 1 mL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 5 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 415 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g of dry weight. Total flavonoid content was calculated from the following calibration curve:

$Y = 0.0067 X + 0.0132$, $R2 = 0.973$
where *Y* is the absorbance of crude extract and *X* is the quercetin equivalent.

2.8. *In vitro antioxidant activity*

Free radicals furnish more than one hundred health disorders in human which may include arthritis, atherosclerosis, ischemia and reperfusion injury of many tissues, injury to central nervous system, CNS, gastritis, cancer and acquired immune deficiency syndrome AIDS [19,20]. Human bodies possesses intrinsic defending

mechanism by neutralizing oxidative stress using its deferent cell components such as glutathione peroxidase, superoxide dismutase, catalase, ubiquinone, glutathione and uric acid [21,22]. The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical DPPH were estimated by the method described by Williams *et al*. [23]. The method is characterized by the reduction of radical DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical DPPH-H. Free radical scavenging potential was portrayed by the transformation of purple color of DPPH in methanol to yellow which is amplified spectrophotometrically. 2.0 mL of MSF and its fractions of the extracts at different concentration were mixed with 3.0 mL of DPPH methanol solution (20 µg/mL). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the extract as compared to ascorbic acid (AA) by UV spectrophotometer. AA was used as positive control. Calculated amount of AA was dissolved in methanol to get a mother solution having a concentration 1000 µg/mL. Serial dilution was made using the mother solution to get differential concentration ranging from 500.0 to 0.977 µg/mL. The reckoned amount of different extractives were taken and dissolved in methanol to get the mother solution (1000 µg/mL). Sequential dilution of the mother solution produced differential concentration. 20 mg DPPH powder was weighed and dissolved in methanol to get a DPPH solution having a concentration 20 µg/mL. The solution was prepared in the amber reagent bottle and kept in the light proof box. 2.0 mL of a methanol solution of the sample (extractives/ control) at different concentration (500 µg/mL to 0.977 µg/mL) were mixed with 3.0 mL of DPPH methanol solution (20 µg/mL). After 30 min reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$(I\ \%) = (1 - A_{sample}/A_{blank})\ X\ 100$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test material). Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration.

2.9. *In vitro thrombolytic activity*

The thrombolytic activity of crude extract and its different organic soluble extractives were evaluated by a method proposed by Daginawala *et al*. [24] using SK as standard substance. 10 mg of methanolic extracts and its different fractions of plant of *P. odoratissimus* were taken in different vials to which 1 mL distilled water was added. Commercially available lyophilized SK of 15, 00,000 I.U., was collected and 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µL (30,000 I.U) was used for the determination of *in vitro* thrombolytic potentials. Aliquots (5 mL) of venous blood samples were collected from

healthy human volunteers by maintaining aseptic condition without a history of oral contraceptive who were distributed in ten different pre-weighed sterile vials (1 mL/tube) and incubated at 37 °C for 45 min. Study protocol was approved by ethical committee of Pharmacy department, World University of Bangladesh. Written consent was obtained from each volunteer prior to collection of blood sample. After clot formation, the serum was completely removed without disturbing the clot and each vial having clot was again weighed to determine the clot weight. To each vial containing pre-weighed clot, 100 µL aqueous solutions of different organic partitionates of *P. odoratissimus* along with the crude extracts was added separately. As a positive control, 100 µL of SK and as a negative non-thrombolytic control, 100 µL of distilled water were separately added to the control vials. All the vials were then incubated at 37 °C for 90 min and observed for clot lysis. After incubation, the released blood serum was removed and vials were again weighed to observe the difference in weight after clot disruption [25,26]. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (Weight of clot after 90 min /Initial weight of clot) × 100

2.10. *In vitro antimicrobial activity*

In vitro antimicrobial activity**,** antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (7 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (Kanamycin 20 µg/disc)) discs and blank discs were used as positive and negative controls. These plates were kept at 4 °C for 24 h to allow maximum diffusion of the test materials to the surrounding media. The plates were then inverted and incubated at 37 °C for 24 h for optimum growth of the organisms. The test materials having antimicrobial property inhibited microbial growth in the media surrounding the discs and thereby yielded a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent was then determined by measuring the diameter of zone of inhibition expressed in mm [27]. In the present study the crude extracts as well as fractions were tested for antimicrobial activity by disc diffusion method.

3. Results and Discussion

3.1. *Phytochemical screening test*

The qualitative exploration of whole plant of *P. odoratissimus* affirmed the existence of different phytochemicals namely tannins, phenols, steroids, flavonoids, alkaloids, saponins and other phytocompounds (Table 1), which may be responsible to generate

the anti-oxidant, anti-microbial, thrombolytic potential activity and other traditional also.

Table 1. Phytochemical screening of methanolic and other organic soluble fraction of *P. odoratissimus.*

Phytochemicals	Remarks
Carbohydrates	++
Reducing sugars	+
Tannins	+++
Alkaloids	++
Flavonoids	++
Saponins	-
Gums	-
Steroids	+
Resins	+++

Depending on the deepness of color; where, += Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount, - = Not present.

The methanolic leaves extract of *P. odoratissimus* and its different organic soluble partitions were subjected to ensure the presence of numerous phytochemicals which are biologically active. Distensible phytochemical, such as carbohydrates, reducing sugars, tannins, alkaloids, flavonoids, saponins, gums, steroids and resins were disclosed in the tested extractives. The obtained phytochemicals in the current experiment are known to be salubrious in medicinal sciences. The knowledge of the current study can be foreseen as decipher in the discovery of new drug molecules [28].

3.2. *Total phenol and flavonoid content*

The probable antioxidant potentials of *P. odoratissimus* were determined by estimation of TPC of methanolic extractives, pet-ether, dichloromethane and aqueous soluble fractions. In this study, MSF showed the highest phenolic and flavonoid content (62.19 ± 0.26 mg of GAE/g, (52.09 ± 0.16 mg of QE/g) of extract respectively while AQSF and DCME displayed the lowest phenolic value (23.44 ± 0.21 mg of GAE/g of extract, 22.99 ± 0.06 mg of QE/g) among all partitionates. But other Kupchan fractionates exhibited considerable amount of phenolic and flavonoid content (Table 2). The existences of notable amount of phenolic compounds mainly flavonoids and other chemicals confirm that the leaf extracts of *P. odoratissimus* are much more pharmaceutically potential. The way of determination of the total phenol and flavonoid content was also done on the basis of absorbance value of crude methanolic extract and it's fractionate. As their free radical scavenging potentiality is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols, isoflavones, flavanonol and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants *in vivo* [29].

3.3. *DPPH free radical scavenging activity*

The crude methanolic extract and various organic soluble factions of *P. odoratissimus* were tested for the evaluation of antioxidant capacity using DPPH and be quantified through a decrease in the maximum absorption of DPPH at 570 nm. A series of test tube was prepared to evaluate the inhibitory concentration of crude extract. The calibration curve was prepared using with AA. The magnitude of IC_{50} of different extractives varied from (36.70 ± 0. 32 µg/mL to 221.31 ± 0.20 µg/mL) (Table 2, Fig. 1). Among the extractives, methanol soluble fraction, MSF exhibited towering free radical scavenging activity (IC_{50} = 36.70 ± 0. 32 µg/mL) in contrast to IC_{50} = 12.48 ± 0.09 µg/mL of AA. Antioxidants are astounding molecules which possess the capability to protect the body tissues from damages caused by oxidative stress. The antioxidant capacity of leaf extracts of *P. odoratissimus* was evaluated in order to isolate the new bioactive antioxidant from natural sources. It was uncovered that leaf extract of *P. odoratissimus* is present with antioxidant compound compared with reference standard vitamin C for DPPH scavenging activity.

Table 2. Total phenolic, total flavonoid contents and free radical scavenging activities of leaves of *P. odoratissimus.*

Sample /Standard	TPC (mg of GAE/g of extract)	Total flavonoid content mg of QE/g extract	DPPH Free radical scavenging activity (IC_{50} µg/mL)
MSF	62.19 ± 0.26	52.09 ± 0.16	36.70 ± 0. 32
PSF	33.44 ± 0.42	42.19 ± 0.26	123.79 ± 0.27
DCMSF	40.31± 0.19	22.99 ± 0.06	86.88 ± 0.71
AQSF	23.44 ± 0.21	32.11 ± 0.16	221.31 ± 0.20
AA (Standard)	-	-	12.48 ± 0.09

Values are means of three biological replicates.

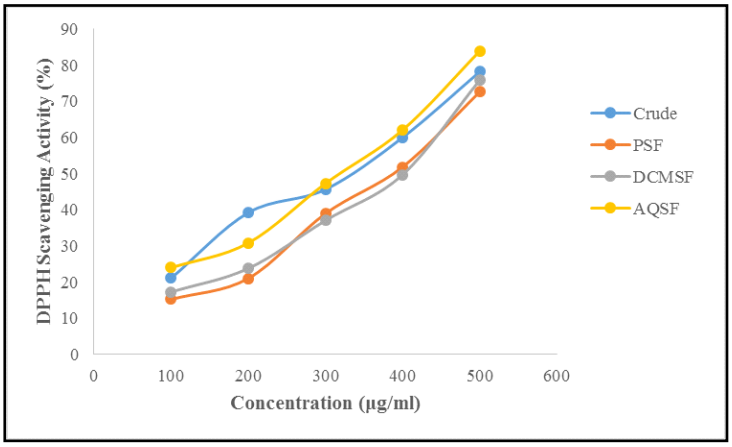

Fig. 1. Dose-response curve of DPPH scavenging activity in the plant leaves of *P. odoratissimu.*

3.4. *Thrombolytic activity*

The crude methanolic and other partitionate of *P. odoratissimus* were subjected to evaluate the probable thrombolytic potentials using SK as positive that demonstrated (69.52 %) lysis of blood clot. On the other conditions, sterile distilled water, a negative control, explored a negligible percentage of clot breaks down (4.24 %). The level, expressed as percent, of clot lysis by various fractions were observed in the following order, AQSF (46.58 %), PSF (40.79 %), DCMSF (39.81 %) and MSF (13.54 %). It may be assumed by the apprehension of the results that (considering > 20 % moderate); ($p < 0.01$; $p < 0.05$) *P. odoratissimus* extract exhibited modest thrombolytic activity as elucidated in Table 3. Thrombosis is a major vascular pathology which is the leading mediator in creating numerous heart ailments especially cardiovascular ischemic events. The present study was aimed to investigate that if there is any thrombus breaking potentiality of leave extracts of *P. odoratissimus.* With the result of positive control (SK), we compared the MSF and its Kupchan fractions at the conc. of 2 mg/mL as the way with the negative control. Our findings suggested that thrombolytic activities of test samples possessed the positive cascades in comparison to positive and negative controls. The findings insinuated that *P. odoratissimus* possessed phytochemicals which are responsible for clot lysis activity.

Table 3. Thrombolytic activity of crude extracts and different organic soluble fractions of leaves of *P. odoratissimus.*

Fractions	Weight of empty vial W_1 (g)	Weight of vial with clot W_2 (g)	Weight of clot $W_3 = W_2-W_1$ (g)	Weight of vial after clot lysis W_4 (g)	Weight of lysis clot $W_5 = W_2-W_4$ (g)	% of clot lysis $=(W_5/W_3)\times100$
MSF	3.78	4.74	0.96	4.61	0.13	13.54 ± 0.63
PSF	3.81	4.57	0.76	4.26	0.31	40.79 ± 0.44
DCMSF	3.88	4.91	1.03	4.50	0.41	39.81 ± 0.32
AQSF	3.71	4.44	0.73	4.10	0.34	46.58 ± 0.21
Negative Control	3.50	4.68	1.18	4.63	0.05	4.24 ± 0.90
Standard (SK)	3.70	4.41	0.71	4.03	0.38	69.52 ± 0.11

Values are means of three biological replicates, the results; ($p < 0.01$; $p<0.05$)

3.5. *Antimicrobial activity*

Antimicrobic experiment was conducted on four gram positive and five gram negative bacteria at a conc. of 400 µg/disc. DCMSF exhibited highest zone of inhibition (19.60 ± 0.12 mm) in *Bacillus subtilis*, a gram positive bacteria. Other organic soluble fractions such as MSF, PSF and AQSF showed moderate antimicrobial activity ranging from (11.50 ± 0.10 mm - 18.30 ± 0.11 mm) respectively compared to the standard antibiotic Kanamycin (20 µg/disc) ranging from (44.00 ± 0.25 mm - 48.00 ±

0.06 mm) of clear zone that characterized the antimicrobial potentials of standard antibiotic. On the other hand, DCMSF faction also displayed the zenithal activity against the growth of *Shigella dysenteriae* (20.00 ± 0.20 mm) which is a gram negative bacteria. Other Kupchan partitionates of *P. odoratissimus* barred the proliferation of bacteria in a moderate spectrum from (12.00 ± 0.41 mm - 18.15 ± 0.11) subsequently in resemblance of standard antibiotic ((42.00 ± 0.23 mm - 50.00 ± 0.19). But PSF did not show any clear zone which characterized that there was no antimicrobial effectiveness in PSF. The results were recorded as presence or absence of clear zone around the disc. The diameter of clear zone indicated the magnitude of antibacterial activity. The events revealed that the variability in diameter of zone of inhibition of each extract against a given bacteria. The outcomes of this research are a bestowal to the valorization of leaf extracts of *P. odoratissimus* which is used in traditional medicine (Table 4.)

Table 4. Antimicrobial profile of different Kupchan fractionates of *P. odoratissimus.*

Test Microorganisms	Diameter of zone of inhibition (mm)				
	MSF	**PSF**	**DCMSF**	**AQSF**	**Kanamycin**
Gram Positive Bacteria					
Bacillus cereus	11.50 ± 0.10	-	18.00 ± 0.10	-	44.00 ± 0.25
Bacillus subtilis	16.30 ± 0.04	-	17.40 ± 0.25	-	47.00 ± 0.18
Staphylococcus aureus	17.85 ± 0.21	-	19.60 ± 0.12	18.30 ± 0.11	48.00 ± 0.06
Sarcinalutea	14.00 ± 0.08	-	13.20 ± 0.20	12.65 ± 0.71	45.00 ± 0.31
Gram Negative Bacteria					
Escherichia coli	12.00 ± 0.41	-	15.00 ± 0.15	-	46.00 ± 0.17
Salmonella typhi	13.00 ± 0.32	-	15.75 ± 0.10	-	45.00 ± 0.09
Shigelladysenteriae	18.15 ± 0.11	-	20.00 ± 0.20	13.00 ± 0.07	50.00 ± 0.19
Vibrio mimicus	12.56 ± 0.22	-	16.00 ± 0.17	-	45.00 ± 0.20
Vibrio parahemolyticus	-	-	13.80 ± 0.25	-	42.00 ± 0.23

'-' no measurable zone; Values are mean ± SEM of 3 replications.

4. Conclusion

This research was strategized in an abridged manner in order to rationalize the leaves of *P. odoratissimus* used as a traditional medicine from primeval age in order to treat numerous health complications such as thrombolytic, membrane stabilizing, antioxidant, cold/flu, asthma, hepatitis, boils, and dysuria. The results of this study uncovered that the leaves of *P. odoratissimus* embodied paramount medicinal properties against formation of thrombus, oxidative stress and both gram positive and negative bacteria. More comprehensive investigation should be carried out in view of isolation and characterization of specific chemical compounds responsible for the previously said medicinal values.

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