



***In vitro* assessment of antibacterial and antioxidant activities of ethanolic leaves extracts of *Paullinia pinnata* Linn (Sapindaceae)**

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ABSTRACT

This study aimed at investigating the *in vitro* antibacterial and antioxidant activities of ethanolic leaves of *Paullinia pinnata*. Antibacterial assay was performed using agar well diffusion and microdilution methods against seven clinical isolates namely *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Shigella flexneri*, *Proteus mirabilis*, *Salmonella typhi* and *Bacillus cereus*. Total phenol content was determined by the Folin-Ciocalteu test while antioxidant activity was evaluated by DPPH, FRAP and ABTS methods. *S. typhi* and *B. cereus* were sensitive to agar diffusion with an inhibition diameter zone of 13.67 ± 1.64 and 13.33 ± 1.43 mm respectively. *S. flexneri* was most sensitive with MIC of 195 µg/ml by microdilution. The total phenol content was found to be 226.748 ± 08.11 mg ascorbic acid equivalent/g of dry material. The scavenging kinetics of DPPH showed a steady state at 60 minutes while EC₅₀ was 0.89×10^3 g/mol in DPPH. FRAP assay showed an absorbance of 0.4 at 50 µg/ml. ABTS test displayed an inhibition percentage of 55.92% at 4.54 µg/ml. These results justify its traditional use to heal infectious diseases. *P. pinnata* leaves are a potential source of antibacterial and antioxidant compounds to be exploited.

Key words: *Paullinia pinnata*, ethanolic extract, antibacterial activity, antioxidant activity

INTRODUCTION

Infectious diseases remain one of the main dangerous diseases in the world and particularly in Africa where the death rate mounts up 45 %. About 90 % of these deaths can be attributed to malaria, AIDS, tuberculosis, measles, acute respiratory tract infections and diarrheal diseases; with 70 % due to infectious bacteria [1]. The major way to fight bacterial infections is by antibiotherapy. However, inappropriate and over use of antibiotics and the insufficient management of infections leads to increase resistance and loss of antibiotherapy. During infection, bacteria like enteroinvasive *Escherichia coli* and *Shigella flexneri* amongst others release lipopolysaccharides and peptidoglycans which stimulate free radicals related to the production of cytokines by macrophages [2-4]. These situations increase lipoperoxydation which leads to degenerative

diseases [5]. However, many synthetic antioxidants such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), tert-Butylhydroquinone (TBHQ) and Propyl Gallate (PG) are used as food additives to prevent lipid peroxidation in many fields [6]. But, these common antioxidants used are suspected to possess toxic effects. Therefore, the search for natural antioxidants as alternatives to synthetic ones is of great interest to researchers. A major way to solve these problems can be the exploration of plants since they are natural sources of biomolecules which are readily available and accessible. Herbal medicines are widely used and now form an integral part of the primary health care in many countries. Significant increase and ameliorations are been noted in phytotherapy but several plants with medicinal properties remain underexploited [7]. Cameroon possess a wide variety of plant species used in traditional medicine in the

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treatment of several infectious diseases and several interesting openings have originated for further inquiry following *in vitro* antimicrobial activity evaluation [8]. *Paullinia pinnata* Linn (Sapindaceae) is a plant used in the Cameroon traditional medicine to heal diarrhea. Aqueous decoctions and powdered leaves from *P. Pinnata* are traditionally used in west Cameroon for the treatment of diarrhea, without strong scientific backings. However, previous works revealed strong antibacterial and antioxidant activities exhibited by *P. Pinnata* [9-12]. The biological properties of *P. pinnata* as well as its traditional usage in the treatment of diarrhea justify further attempts to search for antibacterial and antioxidant chemicals from natural sources. Phytochemical studies of *Paullinia pinnata* entails the isolation of numerous phenolic compounds with biological activities [13-15]. The present study was therefore undertaken to investigate the phytochemical screening and *in vitro* antibacterial activity of ethanolic leaves extracts of *P. pinnata* against clinical isolates causing diarrhea strains as well as its antioxidant activity.

MATERIALS AND METHODS

Collection and identification of plant materials:

P. pinnata plant was collected at Nkoupa-Matapit in the Western region of Cameroon in December 2013. The plant identification was done at the Cameroon National Herbarium in comparison with specimen N° 20022/SRF Cam.

Microorganisms: Seven bacterial species including six Gram negative (*E. cloacae*, *S. typhi*, *E. coli*, *P. mirabilis*, *S. flexneri* and *K. pneumoniae*) and one Gram positive (*B. cereus*), commonly associated with diarrheal infection where used. These clinical isolates were obtained from "Centre Pasteur" of Cameroon and the Yaounde General Hospital. Bacterial strains kept at +4°C were activated before any test.

Preparation of ethanolic extracts: The leaves of *P. pinnata* were air-dried for one week at room temperature and weighed. Each sample was then ground in a mortar and 200 g of the dried powder of each sample was soaked for 48 hours in 600 ml ethanol 95°. The mixing was filtered with Whatman n°1 filter paper and concentrated using a rotary evaporator at 70°C.

Phytochemical screening: Different secondary metabolites such as alkaloid, tannins, saponins, flavonoids, steroids and phenols present in the extracts were revealed using standard methods [16].

Antimicrobial assays

Agar-well diffusion method: The assay was conducted using agar well diffusion method [17], with slight modifications. 100 mg of crude extracts were dissolved in 1 ml of distilled water to obtain a final concentration of 100 mg/ml. The solutions were prepared under sterile conditions in a fume cupboard. 25 µl of gentamicin (40 mg/ml) was diluted in 975 µl of sterile distilled water to a final concentration of 1 mg/ml. The bacterial strains cultured on nutrient agar at 37°C for 18 hours were suspended in sterile distilled water and adjusted to 0.5 McFarland turbidity spectrophotometrically corresponding to bacterial cell concentration of about 1.5×10^8 CFU/ml [18]. Further dilution was made to obtain a final concentration of about 1.5×10^6 CFU/ml. The surface of solidified agar medium in Petri dishes was inoculated with a suspension of the bacterial inoculums using sterile cotton swabs. Wells of 6 mm in diameter were bored on the agar surface and filled with 50 µl of extract (5mg/well) or gentamicin. The Petri plates were left at room temperature for 15 minutes and then incubated at 37°C for 24 hours. The inhibition zones diameter around the well were measured using a vernier caliper. The sensibility of the bacteria strains to the extracts was determined using the inhibition diameter (ID) as: non sensitive ($ID \leq 8$ mm); sensitive ($9 \leq ID \leq 14$ mm); more sensitive ($15 \leq ID \leq 19$ mm) and extremely sensitive ($20 \text{ mm} \leq ID$) [19].

Determination of the Minimal Inhibitory Concentration (MIC) by microdilution method

Preparation of inoculums of microorganisms: A pure colony of bacteria from an overnight culture on Mueller Hinton Agar incubated at 37°C was suspended in a tube containing 1 ml of sterile distilled water. The concentration of the suspension was then standardized as previously described.

Preparation of working solutions of extracts and reference antibiotic: 100 mg of ethanolic extract of *P. pinnata* were weighed and dissolved in 1 ml of sterile distilled water for a final concentration of 100 mg/ml. Reference antibiotic was prepared at the concentration of 0.2 mg/ml.

Preparation of plates: The microdilution was performed in 96-well microtiter plates with U-shaped bottoms as described by Eloff, 1998a [20]. Each well was filled with 100 µl of nutrient broth. 100 µl of each extract was added in the first and a series of two fold dilution of each crude extract was realized until the eleventh well. The standard antibiotic was prepared in the same way. The final concentrations ranged from 50×10^3 to 0.0488 µg/ml for each extract and from 0.1 to 0.09765 µg/ml for the standard antibiotic. The microplates were inoculated with 100 µl of a culture containing 1.5×10^6 CFU/ml of each organism, covered and

incubated for 16-24 hours at 37°C. After incubation, 40 µl of 2,3,5- triphenyltetrazolium chloride (TTC) at 0.01% was added to each well except negative control wells containing only extract and nutrient broth. The microplates were incubated for 30 minutes. The MIC was determined as the lowest concentration of extract inhibiting visible growth of each microorganism in the nutrient broth. Inhibition of bacterial growth in the plates containing extract was judged by comparison with growth in control microplates and by the turning of uncolored TTC to red. Each experiment was realized in triplicate. The third column without TTC was left for determination of minimal bactericidal concentration (MBC). The MBC was determined by pipetting 50 µl from the well without any growth in 100 µl of nutrient broth and incubated at 37°C for 48 hours. After incubation, 40 µl of TTC were added to each well and the plates were incubated at 37°C for 30 minutes. MBC correspond to the lowest concentration of extract at which no visible growth was observed with the naked eyes. The ratio MBC/MIC was calculated to reveal bactericidal/bacteriostatic effects of extract.

Total phenol content: The total phenol content was determined using Folin-Ciocalteu (1/10) diluted reagent as described by Vinson *et al.* (1998) [21]. 23 µl of each extract (1 mg/ml) was introduced in a tube containing 115 µl of Folin-Ciocalteu reagent in 1817 µl of distilled water. After 5 minutes, 345 µl of Na₂CO₃ (15%) solution was added. Ascorbic acid was used as standard. Absorbance was read at 765 nm with a spectrophotometer (Jenway 6305) after 120 minutes of incubation in the dark. Total phenol content was expressed in mg equivalent of ascorbic acid/g of dried extract.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) antiradical assay: DPPH radical scavenging kinetic was carried out as described by Brand-Williams *et al.*, 1995 [22] with slight modifications by measuring the decrease of absorbance each 15 minutes for 2 hours. 50 µl of ethanolic extracts of *P. pinnata* prepared in ethanol at different concentrations (0.8 mg/ml; 0.4 mg/ml; 0.2 mg/ml; 0.1 mg/ml; 0.05 mg/ml; 0.025 mg/ml) was mixed in 1950 µl ethanolic solution of DPPH (0.04 g/l) in different tubes. Final concentrations in 2 ml of DPPH solution were 0.02 mg/ml; 0.01 mg/ml; 0.005 mg/ml; 0.0025 mg/ml; 0.00125 mg/ml. After 120 minutes of incubation in the dark, absorbance was read at 517 nm with a spectrophotometer (Jenway 6305) against a control (containing DPPH + ethanol). Ascorbic acid was used as standard with final concentrations of 0.01 mg/ml; 0.005 mg/ml; 0.0025 mg/ml; 0.00125 mg/ml; 0.000626 mg/ml.

Scavenging activity was calculated using the following formula:

$$\% \text{Scavenging} = (A_{\text{ref}} - A_{\text{mes}} / A_{\text{ref}} - A_{100}) \times 100$$

Where A_{Ref} = Absorbance at t=120 min of control (DPPH solution without antiradical); A_{mes} = Absorbance at t=120 min of DPPH solution with antiradical and A₁₀₀ = Absorbance at the end of reaction = 0.

Scavenging activity of the extracts was expressed as efficient concentration 50 (EC₅₀) (Concentration of sample (g) to scavenge 50% of the DPPH free radicals (mol)) from the linear regression plot. The antiradical power value was also calculated as 1/EC₅₀ (in mol of DPPH/g extract) to express the scavenging activity of extracts.

Ferric Reducing Antioxidant Power Assay (FRAP):

The experiment was carried out as described by Benzie and Strain, 1996 [23]. 3.1 mg of 2,4,6 tripyridyl-1,2,5-triazine (TPTZ) was diluted in 1000 ml of HCl 40 mM. Ferric chloride (FeCl₂) 20 mM and acetate buffer 300 mM, pH 3.6 were mixed in the ratio 1; 1; 10 respectively to obtain FRAP solution. 50 µL of ethanolic extracts of *P. pinnata* prepared in ethanol at different concentrations (2 mg/ml; 1 mg/ml; 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml and 0.0625 mg/ml) were mixed in 1950 µl of ethanolic solution of FRAP in different tubes to a final concentrations of 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml; 0.0625 mg/ml and 0.03125 mg/ml. After 30 minutes of incubation in the dark, absorbance was read at 593 nm with a spectrophotometer (Jenway 6305) against a control. Ascorbic acid was used as the standard.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS) Assay:

Free radical-scavenging activity by ABTS method was performed as described by Re *et al.*, 1999 [24]. The working solution was made up of ABTS 7 mM and potassium persulfate 2.4 mM (1:1, v/v) prepared in distilled water. The solution was left in the dark for 12 hours at room temperature. The resulting solution was diluted by mixing 1 ml of fresh solution of ABTS to obtain an absorbance of 0.706 ± 0.001 read at 734 nm with a spectrophotometer (Jenway 6305). The fresh solution was prepared daily. Extract (20 µl) at different concentrations (31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml and 500 µg/ml) was mixed with 2 ml of ABTS solution to a final concentration of 0.28 µg/ml, 0.56 µg/ml, 1.13 µg/ml, 2.24 µg/ml and 4.54 µg/ml. Absorbance was read at 734 nm after 7 min with a spectrophotometer (Jenway 6305). Ascorbic acid was used as standard. Scavenging activity was calculated as follow:

ABTS radical scavenging activity = $(A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$.

Where A_{control} = Absorbance at t=7 min of control (ABTS solution without antiradical); A_{sample} = Absorbance at t=7 min of ABTS solution with antiradical.

Statistical analysis: Data was represented as mean \pm standard deviation (SD) and subjected to one-way analysis of variance (ANOVA) using the Fisher test at threshold of $p < 0.05$ with Stat graphics plus 5.0 for windows. Excel was used to draw figures

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemical screening of the ethanolic extracts of *P. pinnata* revealed the presence of phenols, tannins, alkaloids, saponins, anthraquinones, steroids, anthocyanins and flavonoids. This result corroborates those of Murutala *et al.*, 2015 [25] who used methanol as the extract solvent. However anthraquinones were absent in the methanolic leaves extract reported by Yusuf *et al.*, 2014 [26] and Paul *et al.*, 2015 [27]. These differences might be ascribed to the nature of compounds in the extract that could differ from the place and season of harvesting and the nature of extraction solvent used. These chemical compounds found in the extract possess antibacterial and antioxidant activities [28].

Antibacterial assay

Determination of the inhibition diameter zones:

The presence of inhibition zones after incubation revealed that the ethanolic extract of *P. pinnata* inhibited the growth of five bacterial strains out of seven tested (Table 1). Among all bacteria tested *K. pneumoniae* (8.33 \pm 1.24 mm) was less sensitive. The resistance of *K. pneumoniae* could be explained by the fact that the outermost layer of *Klebsiella* consists of a large polysaccharide capsule [29]. *B. cereus* (13.33 \pm 1.43 mm), *S. flexneri* (12 \pm 0.81 mm), *P. mirabilis* (12.33 \pm 3.29 mm) and *S. typhi* (13.67 \pm 1.64 mm) were most sensitive. *E. cloacae* was not sensitive with *P. pinnata* and appears less sensitive to gentamicin. According to Moreira *et al.* (2005) [19], *E. cloacae*, *S. flexneri* and *K. pneumoniae* were not sensitive to *P. pinnata* extract while *B. cereus*, *P. mirabilis*, *E. coli* and *S. typhi* were sensitive. All the microorganisms revealed different susceptibility to the ethanolic extract of *P. pinnata* which can be due to the concentration and nature and origin of active compounds in these extracts however the susceptibility depends on the type of extract and the solvent of extraction [29].

Inhibitory parameters: Minimal Inhibitory Concentration (MIC)

P. pinnata inhibited the growth of all bacteria strains tested with MIC ranging between 390.62 (*S. flexneri*, *P. mirabilis* and *K. pneumoniae*) and 1562.5 $\mu\text{g/ml}$ (*E. cloacae*) (table 2). Extracts showed bactericidal activity on *E. coli*, *P. mirabilis* and *E. cloacae* while bacteriostatic activity was observed on *S. typhi*, *K. pneumoniae*, *B. cereus* and *S. flexneri*. These results revealed that these ethanolic leaves extracts are most active than methanolic leaves extract which revealed a MIC from 400 mg/ml on *P. mirabilis*, *P. aeruginosa*, *S. aureus* and *A. baumannii* while *E. coli* was not sensitive as revealed by Ikhane *et al.*, (2015) [30]. The MIC obtained with *S. typhi* (781.25 $\mu\text{g/ml}$) corroborates those of Lunga *et al.*, (2014) [12] while the same author found that *E. coli* and *K. pneumoniae* were more sensitive with methanolic leaves extract. These results can be explain by the fact that methanol was found to be the best extracting solvent since it possess ability to make soluble and enhance the extraction of a large quantity of secondary metabolites due to its polarity, concentration or nature [31]. *K. pneumoniae* which was less sensitive in solid medium appeared most sensitive to microdilution while *E. cloacae* which do not sensitive appears sensitive in liquid medium even if it has the higher MIC. This sensitivity in liquid medium can be due to the fact that the extracts are directly in contact with microorganisms, hence the inhibitory potentials increased. The different sensitivities of bacteria to plant extracts can be explained by the fact that bacterial strains may have some kind of resistance mechanisms like enzymatic inactivation, target sites modification and decrease in intracellular drug accumulation [32]. Moreover, the mechanisms of action of the active ingredient can vary from one strain to another and from one species to another and depend equally on the genetic material of each microorganism [33]. The MBC/MIC ratio revealed that except *K. pneumoniae* which is the less sensitive bacteria amongst all bacteria tested *P. pinnata* ethanolic leaves extracts exhibited bactericidal activity. This bactericidal activity justifies the use of this plant to fight infectious diseases. The antimicrobial activity of the extracts can be attributed to the presence of bioactive metabolites present in plants [34]. Previous works revealed that polyphenols inhibit a wide range of microorganisms by specific reaction mechanism with proteins related polyamides polymers [35]. Flavonoids have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [36]. Alkaloids are nitrogen-containing naturally occurring compound, commonly found to have antimicrobial properties due to their ability to insert in DNA of

the microorganisms. The phytochemicals identified in *P. pinnata* leaves could therefore be responsible for antibacterial activity observed since they can together react by synergy.

Antioxidant activity

Total phenol content: Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Total phenol content was determined from the linear calibration curve of ascorbic acid within range of 0.00125-0.01 mg/ml ($Y = 20.21x - 0.024$; $R^2 = 0.957$) as shown in figure 1. The total phenol content was found to be 226.748 ± 08.11 mg ascorbic acid equivalent/g of dry material. The total phenol content was very close to those found in the literature [9]. A strong correlation between phenol and antioxidant activity of plant extracts was demonstrated [37].

Ferric Reducing Antioxidant Power Assay (FRAP):

The reducing capacity of a given compound is based on the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form, and the yellow test solution changing to green and blue depending on the reducing power of each compound [38]. The reducing power was expressed in terms of absorbance at different concentrations of *P. pinnata* extracts and ascorbic acid (Figure 2). From the regression plot (ascorbic acid : $Y = 0.036x + 0.090$, $R^2 = 0.996$), the reducing power of ethanolic leaves extracts was expressed in terms of mg ascorbic acid equivalent /g of dry material. The FRAP assay shows an absorbance of 0.4 at 50 μ g/ml and a reducing power of 3.303 mg ascorbic acid equivalent/g of dry material. However, methanolic extract of *P. pinnata* was found to possess moderate reducing power [9].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay:

DPPH is a stable free radical that shows maximal absorbance at 517 nm in ethanol. When DPPH encounters a proton-donating substance (H^+), the radical is trapped by changing color from purple to yellow and the absorbance is reduced. Thus, in this study, *P. pinnata* reduced the DPPH radical to a yellow-colored compound due to the DPPH radical accepting an electron or hydrogen ion to become stable [39]. Based on this principle, the antioxidant activity of a substance can be expressed as its ability to scavenge the DPPH radical [40].

Scavenging kinetic of DPPH: The scavenging kinetic of the reactivity of DPPH was evaluated. It's aimed at evaluating the reactivity of DPPH at various times with different test compounds by measuring their absorbance every 15 minutes for 120 minutes. There are three types of scavenging

kinetic corresponding to the time at which steady state is obtained namely fast (<10 minutes), intermediate (20 minutes) and slow (>20 minutes) [6]. The kinetic of ethanolic extract of *P. pinnata* (figure 3) and ascorbic acid (figure 4) showed a decrease in absorbance with increasing time. The evaluation of DPPH reactivity revealed that ethanolic extract of *P. pinnata* possess slow scavenging kinetic corresponding to the steady state obtained after 60 minutes. The reference antioxidant showed the same result. However, evaluation of different kinetics depends on the nature of the tested antiradicals [6].

Antiradical activities: The DPPH radical-scavenging capacity in this study was reported after 120 minutes of reaction. The radical scavenging activity of the extract and reference was expressed in terms of efficient concentration (EC_{50}) value which is the concentration of antioxidant required for 50% scavenging of DPPH radicals during the reaction. The smaller the EC_{50} value, the higher the antioxidant activity of the plant extract/reference. The results were expressed in terms of scavenging percentage at various concentrations of extracts and ascorbic acid (Figure 5). It can be noticed that at concentrations less than 5 μ g/ml, no significant difference was observed between scavenging percentage exhibited by *P. pinnata* and ascorbic acid. The reverse effect was observed at concentrations higher than 5 μ g/ml. From the above plot (*P. pinnata*: $Y = 5.725x + 2.447$, $R^2 = 0.968$ and ascorbic acid: $Y = 9.816x - 9.362$, $R^2 = 0.987$), the EC_{50} of ethanolic extract of *P. pinnata* was found to be 0.89×10^3 g/mol in DPPH obtained from SC_{50} of 8.30×10^{-3} g/l which were not in accordance with those of Annan *et al.*, 2009 [10] who obtained an IC_{50} value of 3.8 μ g/ml with methanolic crude extract of *P. pinnata*. The results obtained by Jimoh *et al.*, 2007 [9] showed that methanolic crude extract of *P. pinnata* possess strong scavenging activity. The antiradical power of *P. pinnata* was found to be 1.122×10^{-3} mol/g. At the time, ascorbic acid shows an EC_{50} of 0.577×10^3 g/l with an antiradical power of 1.732×10^{-3} mol/g.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical scavenging activity :

ABTS radical assay is a widely used method to measure antioxidant activity in which the radical is trapped to form ABTS radical complex [41]. The $ABTS^{\bullet+}$ is a relatively stable radical that is easily reduced by an antioxidant [42]. The scavenging capacities of extracts and ascorbic acid for the ABTS radical were measured by evaluating the inhibition percentage at different concentrations of extracts (Figure 6). As in the case of DPPH radical scavenging, the scavenging effect of both extract and ascorbic acid increased with increasing

concentration. *P. pinnata* extract exhibited an inhibition percentage of 55.92% for the ABTS radical at 4.54 µg/ml in accordance with Jimoh *et al.*, 2007 [9] whose result showed that methanolic crude extract of *P. pinnata* possess strong scavenging activity by ABTS assay. At the same concentration, ascorbic acid exhibited an inhibition percentage of 63.16%.

CONCLUSION

The phytochemical screening of the ethanolic crude extracts of *P. pinnata* revealed the presence of active chemical compounds (phenols, tannins, alkaloids, saponins, anthraquinones, steroids and flavonoids) with antibacterial properties. The antibacterial assay shows that crude extracts of *P. pinnata* inhibited the growth of all bacterial strains tested. Bactericidal activity of *P. pinnata* leaves extracts was observed on *S. typhi*, *E. cloacae*, *P. mirabilis* and *E. coli*. The results obtained showed that *P. pinnata* leaves extract contain an important

amount of total phenol responsible for its remarkable antioxidant activity exhibited. These results justify the use of *P. pinnata* leaves in Cameroon traditional medicine to heal infectious diseases especially diarrhea and it can also be considered as an important reservoir of antioxidant biomolecules to be exploited. Further studies are required for *in vivo* antidiarrheal activity, the isolation and characterization of antibacterial and antioxidant compounds as well as their toxicity.

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Conflict of Interest: The authors have none to declare.

Table 1: Inhibition Zone diameter of ethanolic leaves extracts of *P. pinnata* and gentamicin

Tests Substances	Inhibition Zone (mm)						
	ST	KP	BC	EC ₁	SF	PM	EC ₂
<i>P. pinnata</i>	13.67± 1.64	08.33± 1.24	13.33± 1.43	10.66± 1.69	12±0.81	12.33± 3.29	00±00
Gentamicin	25.83± 0.62	24.5 ± 2.67	22.33± 0.94	21.5 ± 0.40	29 ± 0.81	29.55± 0.42	17.66 ± 0.47

ST: *S. typhi*; KP: *K. pneumoniae*; BC: *B. cereus*; EC₁: *E. coli*; PM: *P. mirabilis*; EC₂: *E. cloacae*; *S. flexneri*. Inhibition zone is expressed as mean ± standard deviation (SD) of three replicates.

Table 2: Minimum Inhibitory Concentration (MIC) of ethanol extracts of *P. pinnata* and gentamicin

Extract/reference (µg/ml)	Inhibition parameters	ST	KP	BC	EC ₁	SF	PM	EC ₂
<i>P. pinnata</i>	MIC	781.25	390.62	781.25	781.25	195	390.62	1562.5
	MBC	3215	3215	3215	1562.5	781.25	390.6	3215
	MBC/MIC	4	8	4	2	4	1	2
Gentamicin	MIC	0.390	0.390	0.195	3.125	0.390	12.5	0.781
	MBC	0.781	0.781	0.390	12.5	0.390	25	1.562
	MBC/MIC	2	2	2	4	1	2	2

ST: *S. typhi*; KP: *K. Pneumoniae*; BC: *B. cereus*; EC₁: *E. coli*; PM: *P. mirabilis*; EC₂: *E. cloacae*; SF: *S. flexneri*

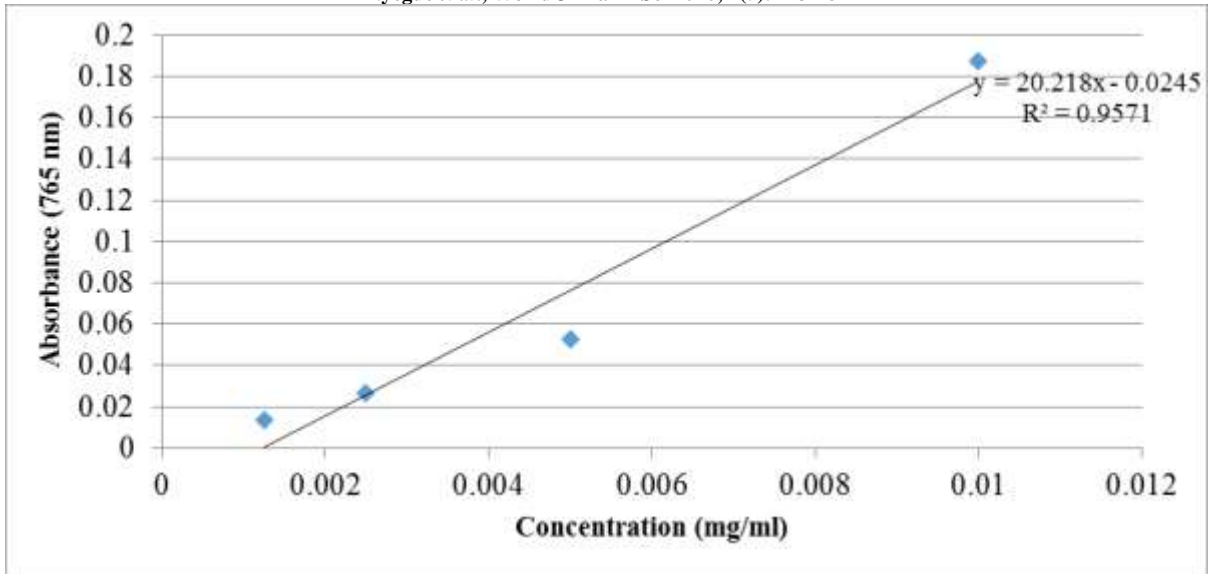


Figure 1: Calibration curve of ascorbic acid for determination of total phenol content

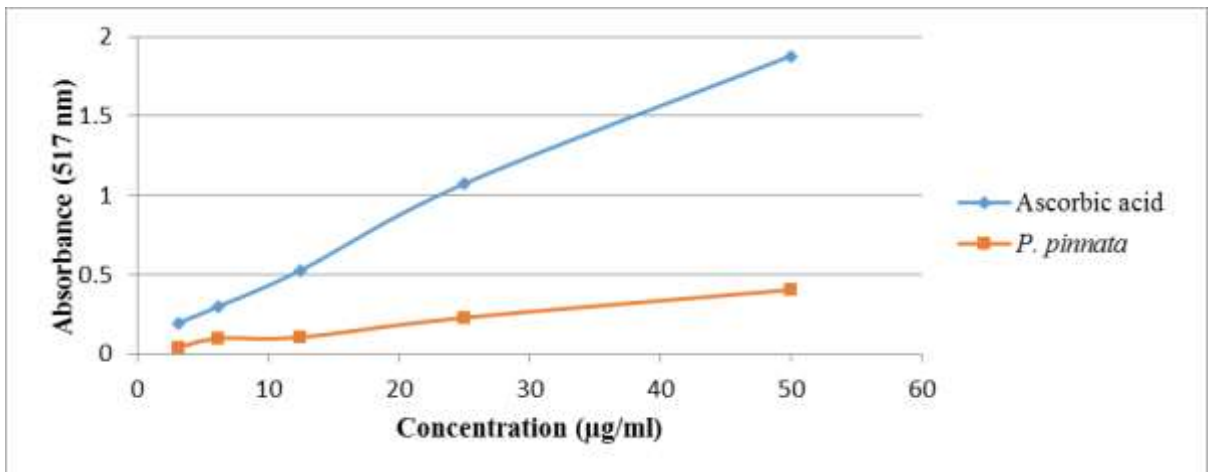


Figure 2: Ferric reducing antioxidant power (FRAP) at different concentrations (3.12–50 µg/ml) of ethanolic extract of *P. pinnata* and reference antioxidant

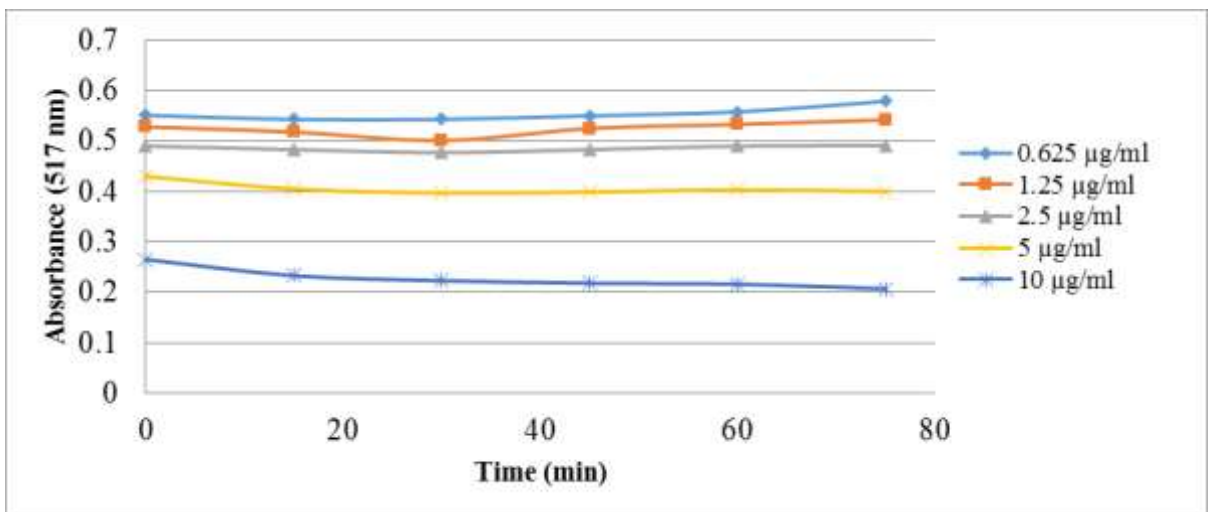


Figure 3 : Scavenging kinetic of *P. pinnata* in terms of absorbance at different times

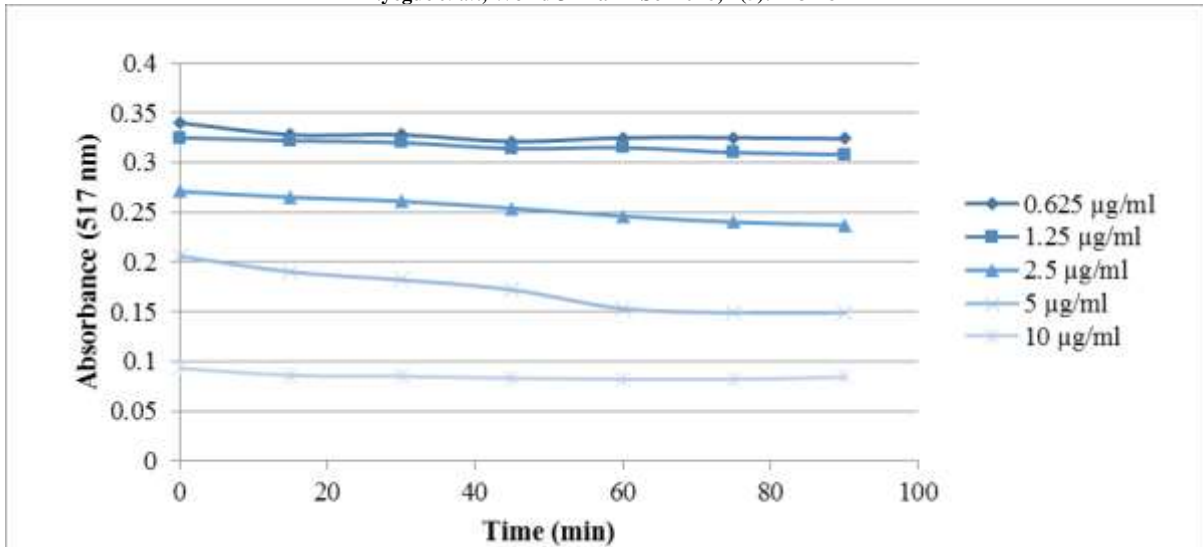


Figure 4: Scavenging kinetic of ascorbic acid in terms of absorbance at different times

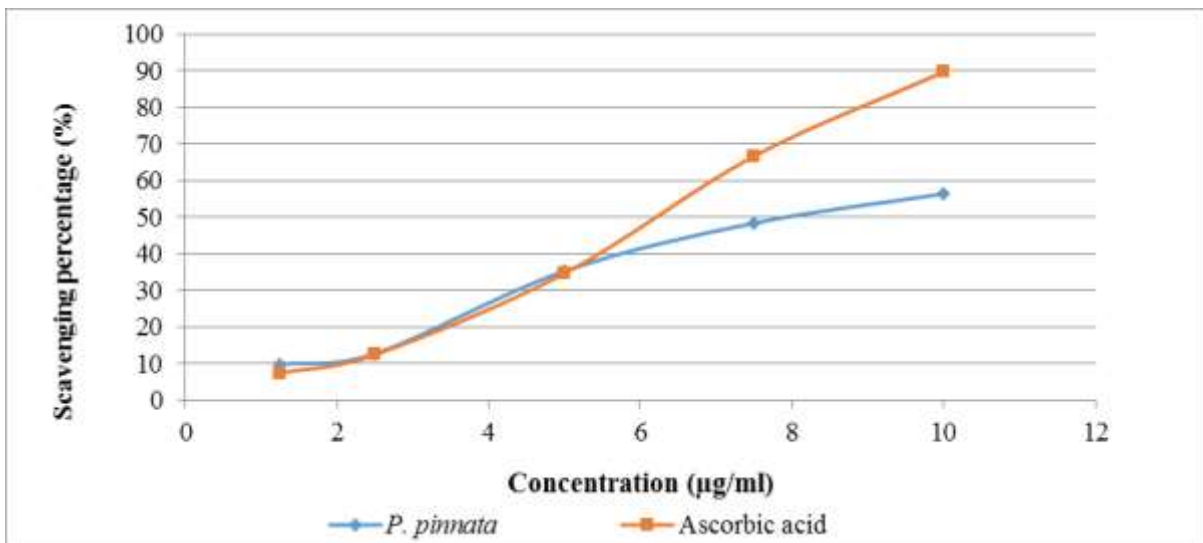


Figure 5: DPPH free radical scavenging activity of different concentrations (1.25–10 µg/ml) of ethanolic extract of *P. pinnata* and reference antioxidant

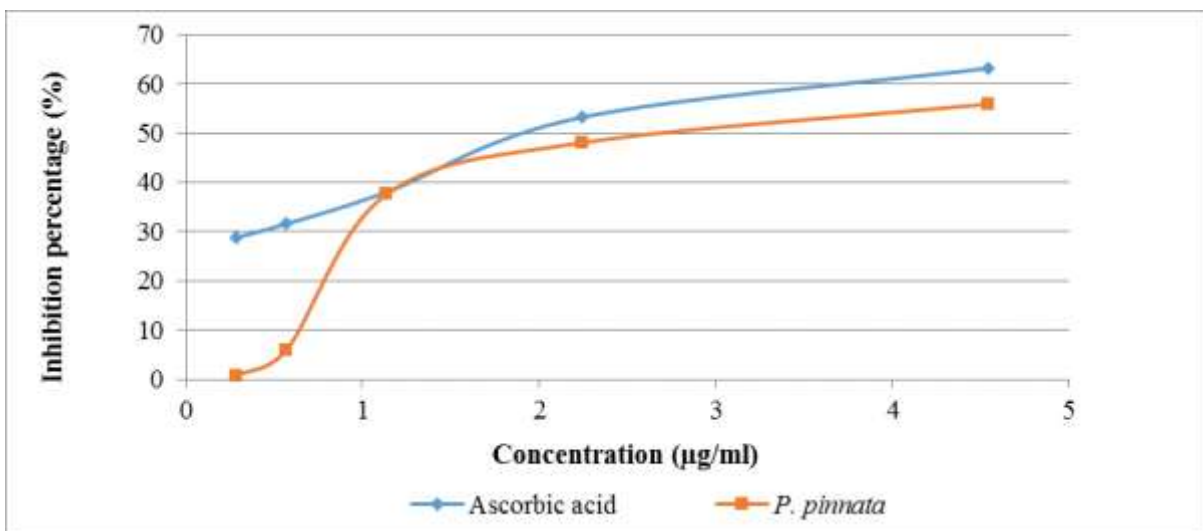


Figure 6: ABTS radical scavenging activity of different concentrations (0.284–4.54 µg/ml) of ethanolic extract of *P. pinnata* and reference antioxidant

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