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Preliminary Antimicrobial and Antioxydant Activities of Extracts of *Pseudospondias microcarpa*

N. Nnanga[1,2,3], Y.T. Omgba[2], S.A. Ngué[3], C. Mbakop[1], L.S. Sidjui[1,4*], L.N. Famen[3], Y.A.T.Ngandjui[4], R.M. Kouipou Toghueo[5], Y.O. D. Nganso[6], E.M. Mpondo[3]

[1]Institute of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon
[2]Department of Galenic Pharmacy and Legislation, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon
[3] Department of Pharmacy, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon
[4] Department of Organic Chemistry, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon
[5] Department of Biochemistry, Faculty of Sciences University of Yaoundé I, Yaoundé, Cameroon
[6] Department of Chemistry, Faculty of Sciences, University of Maroua, Maroua, Cameroon
**lazaresidjui@yahoo.fr*

Abstract: *There is a need for new, effective and affordable drugs to fight against infectious diseases in the world. Pseudospondias microcarpa, widely used in the traditional African medicine could serve in a search for plant-derived antimicrobial agents. Thus, this study aimto evaluate antimicrobial and antioxidant activities of extracts of Pseudospondias microcarpa (Anacardiaceae). The phytochemical screenings of extracts was done follow by their antioxidant activity by DPPH and FRAP techniques. The total polyphenols content content was determined. The antimicrobial activity was screen by disc diffusion method and the Minimal inhibitory concentration of selected extract was determined by broth microdilution method. Phytochemical screening showed the presence of sterols, alkaloids, flavonoids, cardiac glycoside, saponins, quinones, glycosides, polyphenols, triterpenes, anthocyanins, anthraquinons, tannins and coumarins. The ethyl acetate of root exerted the best radical scavenging activity being with IC_{50} equal to8.6013±1.04469 µg/mL. The reducing power was ranging from 0.15 ±0.05 to 2.38 ±0.00DO/µg with the hydroalcohol extract of stem bark exhibiting the best ferric reducing antioxidant power. For total phenol content, hydro-ethanol extracts of stem bark was the most riche extract with 3.594 mgEAG/mg.The significant degree of inhibitory activity of hexane extract of fruit and ethyl acetate extract of roots was observed against Salmonella enterica follow by Enterococcus feacalis 51229 and Staphylococcus aureus BAA 977 with respective inhibition diameter of 16.0 mm and 15.0 mm. The MIC determination of selected extracts showed the results range from 156.25 to 2500µg/mL. The ethyl acetate extract ofstem bark was the most active against Staphylococcusaureus 29213 and Staphylococcus aureus BAA 977 with MICs values of 156,25 µg/mL and 312,5 µg/mL respectively. These results support the traditional usage of P. microcarpa by local communities as the therapeutic agents for the treatment of infection disease.*

Keywords: *Pseudospondias microcarpa, Antimicrobial, Antioxidant, Phytochemical screening*

1. INTRODUCTION

The need for new, effective and affordable drugs to treat microbial diseases in the developing world is one of the issues facing global health today [1].In fact, the discovery of antibiotics has decreased the spread and severity of a wide variety of diseases. However, their uncontrolled use has increased the emergence of microbial resistance strains that reduce their efficacy [2]. In addition, the generation of reactive oxygen species (ROS) during the infection process has been implicated in the etiology of over one hundred human diseases [6].The increased production of these toxic oxygen derivatives is considered a universal feature of stress conditions [7]. Antioxidants able to neutralize these ROS and their actions are considered beneficial [1].Due to these reasons, there is a urgent need to identify new antimicrobial agents that would help in alleviating the problems of emerging resistant strains and would act as antioxidant agents.

Medicinal plants are of great importance to the health of human's beings. Thus, a systematic search for useful bioactivities compounds from medicinal plants to fight against microbial infection is now

considered to be a rational approach in nutraceutical and drug research. Thus, *P. microcarpa* a medicinal plant used for the management of various diseases including CNS disorders, arthritis, rheumatism, eye problems, kidney disorders, naso-pharyngeal infections, stomach complaints, malaria and jaundice can be a source of new drugs [11, 12]. Thus, the present work aimed to study antimicrobial and antioxydant activities of extracts from root, stem barks and fruits of *P. microcarpa*.

2. MATERIAL AND METHODS

2.1. Collection of Plant Material

Plant materials were harvested in Yaoundé (October 2014) and identified as *P. microcarpa* at the Cameroon National Herbarium (HNC) where voucher specimens (41437/HNC) are deposited. Then, the stem barks, roots, fruits and leaves were collected and cut in small pieces, dry at home temperature and powdered.

2.2. Extraction Procedure of Plant Material

600g of dried and ground root, stem barks, fruits of *P. microcarpa* were exhaustively macerated successively with *n*-Hexane (n-Hex, 3 L), ethyl acetate (AcOEt, 3 L), alcohol-water (7:3, *v/v*) (EtOH-H2O, 3 L), then (200 g) of each air dried and powdered were exhaustively macerated with water (H2O, 2 L) and ethanol-water (7:3, *v/v*) (EtOH-H2O, 3 L) respectively at room temperature for 48 h. The macerate was filtered and evaporated under reduced pressure to obtain crude extracts labelled PMR corresponding to roots, PMEt corresponding to stem barks, PMFr corresponding to fruits and PMFe corresponding to leaves.

2.3. Phytochemical Screening

The extracts were subjected to phytochemical screening to detect the presence of alkaloids, tannin, saponins, flavonoïds, glycosids, sterols, triterpens, anthraquinone, phenols, cardiac glycosids, quinones, anthocyanins, coumarins and polyphenols using protocols described by Harbone [16].

2.4. Antioxidant Activity

2.4.1. Free Radical Scavenging Activity: DPPH Test

Antioxidant activity of extracts was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Mensor et al [17]. Briefly, 100 µL of extract/fraction prepared at 2000µg/mL were serially diluted and mixed with 900 µL of 0.3 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) , to give five concentrations range from12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL). After an incubation period of 30 min at 25°C, the absorbances at517 nm (the wavelength of maximum absorbance of DPPH) were recorded as $A_{(sample)}$.A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as $A_{(blank)}$. The free radical-scavenging activity of each solution was then calculated as percent inhibition according tothe following equation:

RSA = [(Absorbance of DPPH - Absorbance of sample)/Absorbance of DPPH] x 100.

The radical scavenging percentages were plotted against the logarithmic values of the concentration of test samples and a linear regression curve was established in order to calculate the RSA_{50}, which is the amount of sample necessary to inhibit by 50 % the free radical DPPH. Antioxidant activity of the extracts was expressed as RSA_{50} defined as the concentration of the test material required to cause a 50 % decrease in initial DPPH concentration. Ascorbic acid was used as a standard. All measurements were performed in triplicate.

2.4.2. Ferric Reducing Antioxidant Power

The ferric reducing power was determined as reported by [19].Briefly,400, 200, 100, 50, and 25 µL of solution of methanolic extracts (2000 µg/mL) were mixed with 500 µL of phosphate buffer (pH 6.6) and 500 µL of 1% potassium ferricyanide and incubated at 50°C for 20 min. Then 500 µL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Supernatant (500 µL) was diluted with 500 µL of water and shaken with 100 µL of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Vitamin C and BHT were used as a positive control.

2.4.3. Phenol Content

The total phenolic compounds were determined as described by [18] with slight modification. The reaction mixture consisted of 0.02 mL of extracts and fractions (2 µg/mL), 0.02 ml of 2N FCR (Folin Ciocalteu Reagent) and 0.4 mL of a 20% sodium carbonate solution. After 20min of incubation at room temperature the absorbance was measured at 760 nm.Distilled water was used as control. A standard curve was plotted using Gallic acid (0-0.2 µg/mL).All measurements were performed in triplicate.

2.5. Antimicrobial Assays

2.5.1. Test Microorganisms

The microorganisms used in this study were strains and isolates respectively provided by American Type Culture Collection and University Health Center. They were:

Staphylococcus aureus ATCC 29213 and *BAA 977, Enterococcus feacalis ATCC51299, Aerococcus viridans ATCC 11563, Neisseria gonnorhea ATCC 49226, Escherichia coli ATCC 11775, Pseudomonas aeruginosa ATCC 9027* and *ATCC 10145, Klebsiella pneumonia ATCC 700603, Salmonela enterica, Proteus mirabilis,* and *Candida albicans*.

2.5.2. Preparation of Extracts Stocks and Disc Solutions

For the antimicrobial activity, stock solutions of plant extracts were prepared at 150 mg/mL in DMSO10 %.Ciprofluxacin, gentamicin and ketoconazol were prepared in the same conditions. For disc preparation, 15µL of each stock solution was dropped onto sterilized paper disks(6 mm diameter) and dried at room temperature for a final concentration of1.500 µg / disc.

2.5.3. Antibacterial Screening of Plants Extracts by Disk Diffusion Method

In vitro antimicrobial activity was screened by disc diffusion method using Mueller Hinton Agar (MHA) obtained from Mast Group Ltd. The MHA plates were prepared by pouring 15 mL of molten media into sterile plates (90 mm). The plates were allowed to solidify for 5 min and 0.1 mL of inoculum suspension was swabbed uniformly and the inoculum allowed to dry for 5 minutes.

The different extracts and referents drugs loadedat 1.500 µg /discs were placed on the surface of the medium and allowed to diffuse for 5 min. The plates were incubated at 35 °C for 24 hours for bacteria and for 48 hours for yeast. Negative control was prepared using 10 % DMSO. Ciprofluxacin, gentamicin and ketoconazol were used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with a Vernier Calliper in millimeter. Each experiment was performed in triplicate [20]**.**

The activity of extracts was classified as follows: 10 - 12 mm low activity; 13 – 17 mm moderate activity and > 17 mm high activity [21].

2.6. MICs and MBC/MFCs Determination

The MIC was determined by microdilution method according to Clinical laboratory Standards Institute (CLSI) M27-A3 for yeast and M38 for bacteria, using (12 x 8 wells) microtitre plates (CLSI, 2008). In the well of the plate, 100 µL of Mueller Hinton Broth for bacteria and Sabouraud Dextrose Broth for yeast were introduced. Later on, 100 µL of stock solution of extracts/fractions at 80mg/ml were added to the first well and mixed thoroughly before transferring 100 µL of the resultant mixture to the well of the second line. Serial two-fold dilutions of the test samples were made and 100 µL of inoculum standardized at 0.5 Macfarland for bacteria and at 2.5×10^3Cells/ml for yeast were introduced in the entire well containing the test substances except the column of blank which constitute the sterility control. The concentrations ranged from20 mg/ml to 0.15625 mg/ml for extracts and from 5 µg/mL to 0.195 µg/mL for nystatin and Ciprofloxacin. After the incubation period at 37°C for 48 hours for yeast and 24 hours for bacteria, turbidity was observed as indication of growth. Thus, the lowest concentration without turbidity was considered as the MIC. The MFCor MBC were determined by transferring 50µL aliquots of the clear wells into 150 µL of freshly prepared broth medium and incubated at 37°C for 48 and 24 hours. The MFC and MBC were regarded as the lowest concentration of test sample which did not produce turbidity, indicating no microbial growth. All tests were performed in triplicates. The bactericidal and fungicidal effects were determined by calculating the CMB or MFC / MIC ratio [22].

The classification of extracts of plant material on the basis of CMI is as follows: - strong inhibition: MIC<500 mg/mL; - Moderate inhibition: MIC from 500 mg/mL to 1500 mg / mL; - Weak inhibition: MIC>1500 mg/mL [23].

2.7. Statistical Analysis

Data were statistically analyzed using the software SPSS 17.0 for windows and variance analysis by ANOVA coupled with Waller-Duncan test where $P<0.05$ was considered as statistically significant.

3. Results and Discussion

3.1. Phytochemical Content of Plant Extracts

The qualitative phytochemical screening of crude extracts showed the results presented in table 1. From these results, a number of metabolites classes were identified in all the plant extracts among which sterols, alkaloids, flavonoids, cardiac glycoside, saponins, quinones, glycosides, polyphenols, triterpenes, anthocyanins, anthraquinons, tannins and coumarins. These results are in agreement with finding of many authors who identified the same chemicals classes in extracts of root, stem-barks and leaves of *P. microcarpa* [13, 14, 24].

3.2. Antioxidant Assay

The results for DPPH radical scavenging activity are presented in figure 1, 2 and table 2. All the extracts showed scavenging effects at various levels, with radical-scavenging activity (RSA) ranging from 24.05 ±0.87% to 97.84 ±0.80%. Ethyl acetate extract of fruit exerting the least effect (RSA=24.05 %) and hydro-ethanolic extract of stem bark being the most active (RSA=97.84 ±0.80 %).

Table2. *IC50 values of extracts of Pseudospondias microcarpa.*

EXTRAITS	IC_{50}(µg/mL)
PMR ACOET	8,6013±1,04469[a]
PMR HY-ET	9,7270±0,06600[c]
PMR HY-ETb	9,7200±0,10068[c]
PMEt ACOET	9,3023±0,06929[b c]
PMEt HY-ET	9,7643±0,04692[c]
PMEt HY-ETb	9,2963±0,04484[b c]
PMFr ACOET	14,5237±0,36001[e]
PMFr HY-ET	46,2770±0,85563[f]
PMFr HY-ETb	35,7837±0,70016[g]
PMFe HY-ETb	9,6420±0,04157[c]
VIT C	8,6696±0,02107[a b]
BHT	10,6456±0,10155[d]

Stem back extract: PMEt ACOET = ethyl acetate, PMEt HY-ET = hydro-ethanolic crude; PMEt H20 = Aqueous, PMEt HY-ETb = hydro-ethanolic crude. Root: PMR ACOET = ethyl acetate, PMR Hy-ET = hydro-éthanolic, PMR HY-ETb = hydro-ethanolic crude. Fruit: PMFr ACOET = ethyl acetate, PMFr Hy-ET = hydro-ethanolic, PMFr H20 = Aqueous, PMFr HY-ETb = hydro-ethanolic crude. Leaves:PMFe HY-ETb = hydro-éthanolic crude. Vit C= Vitamin C, BHT: Butylhydroxytoluene

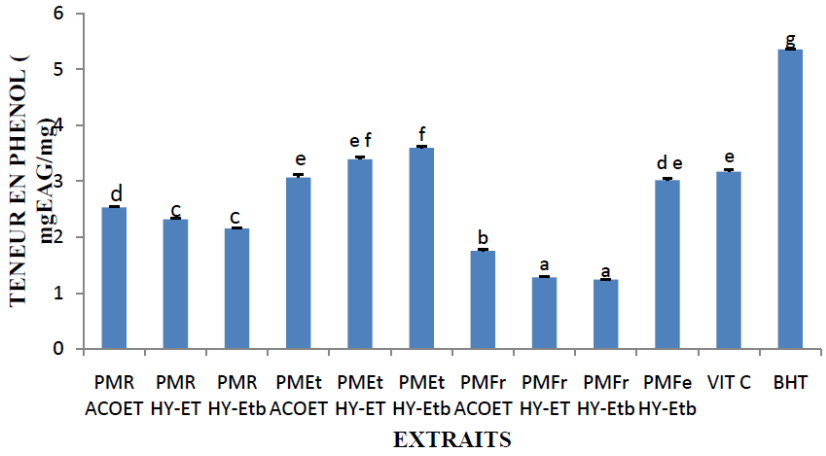

Figure1. *Free polyphenolic concentration of plants extracts as determined using Folin reagent.*

Stem back extract: PMEt ACOET = ethyl acetate, PMEt HY-ET = hydro-ethanolic crude; PMEt H20 = Aqueous, PMEt HY-ETb = hydro-ethanolic crude. Root: PMR ACOET = ethyl acetate, PMR Hy-ET = hydro-éthanolic, PMR HY-ETb = hydro-ethanolic crude. Fruit: PMFr ACOET = ethyl acetate, PMFr Hy-ET = hydro-ethanolic, PMFr H20 = Aqueous, PMFr HY-ETb = hydro-ethanolic crude. Leaves:PMFe HY-ETb = hydro-éthanolic crude. Vit C= Vitamin C, BHT: Butylhydroxytoluene

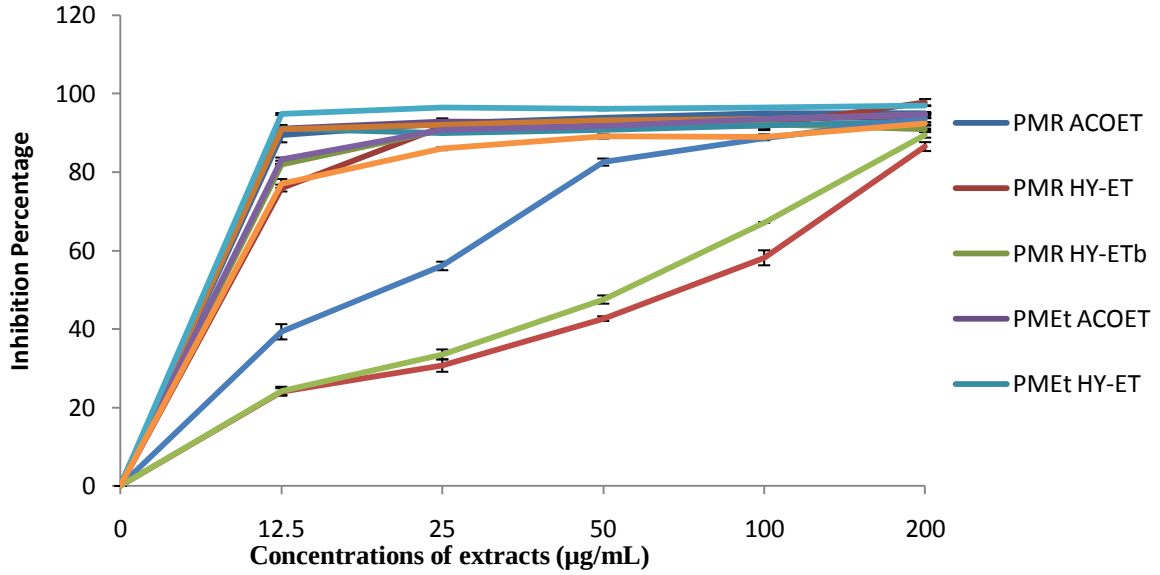

Figure2. *Radical scavenging activity of extracts of P. microcarpa*

Stem back extract: PMEt ACOET = ethyl acetate, PMEt HY-ET = hydro-ethanolic crude; PMEt H20 = Aqueous, PMEt HY-ETb = hydro-ethanolic crude. Root: PMR ACOET = ethyl acetate, PMR Hy-ET = hydro-éthanolic, PMR HY-ETb = hydro-ethanolic crude. Fruit: PMFr ACOET = ethyl acetate, PMFr Hy-ET = hydro-ethanolic, PMFr H20 = Aqueous, PMFr HY-ETb = hydro-ethanolic crude. Leaves:PMFe HY-ETb = hydro-éthanolic crude. Vit C= Vitamin C, BHT: Butylhydroxytoluene

Table1. *Results of phytochemical screening*

	A	S	F	T	CR	P	TT	ST	C	R
PMR HEX	-	-	+	-	-	-	-	+	+	-
PMR ACOET	+	+	+	+	-	+	-	+	+	-
PMR HY-ET	-	+	+	-	-	+	+	+	-	-
PMR HY-ETb	-	+	+	-	-	+	+	+	-	-
PMR H20	-	+	-	-	-	-	-	-	-	-
PMEt HEX	-	-	-	-	-	+	+	-	+	-
PMEt ACOET	+	+	+	+	-	+	-	+	+	+
PMEt HY-ET	-	+	+	+	+	+	+	+	+	-
PMEt HY-ETb	-	+	+	+	+	+	+	+	+	+
PMEt H20	-	+	+	+	-	+	-	-	-	+
PMFr HEX	-	-	-	+	+	+	+	+	+	+
PMFr ACOET	+	+	+	+	+	+	-	+	-	-
PMFr HY-ET	-	+	+	-	+	+	+	-	+	-
PMFr HY-ETb	-	+	+	-	+	+	+	-	+	+
PMFr H20	-	+	+	+	+	-	-	-	-	-
PMFe HY-ETb	+	+	+	+	+	+	+	+	+	-
PMFe H20	-	+	+	-	+	-	-	-	-	-

A:Alkaloids, S:Saponosides, F:Flavonoids, T:Tannins, CR:COMPOSE reducteurs, P: Polyphenol, TT: Triterpenes, ST: Steroids, C:Coumarins, R:RESINS. +: Presence, -: Absence

Stem bark extracts: PMEt-HEX = hexane, PMEt-ACOET = ethyl acetate, PMEtHY-ET = hydro-ethanolic crude; PMEt H20 = Aqueous, PMEt HY-ETb = hydro-ethanolic; Fruit extarcts : PMFr HEX = hexane, PMFr-ACOET = ethyl acetate, PMFr Hy-ET= hydro-ethanolic crude, PMFr HY-ETb = hydro-ethanolic, PMFr H20 = Aqueous; Root extrats: PMR-HEX = hexane, PMR-ACOET = ethyl acetate, PMR H20 = Aqueous, PMR HY-ETb = hydro-ethanolic; Leaves: PMFe H20 = Aqueous, PMFe HY-ETb= hydro-ethanolic.

From table 3, the IC_{50} were range from 8.6013±1.04469 µg/mL to 46.2770±0.85563µg/mL. hydro alcohol extract of root was the lest active (46.2770±0.85563 µg/mL) while the most active extract was ethyl acetate of root (8.6013±1.04469 µg/mL). At the tested concentration, the activity of these extracts was significantly ($P<0.05$) higher than those of ascorbic acid (8,6696±0,02107) and BHT(10,6456±0,10155).

Table3. *Diametres d'inhibitions of extract from p. microcarpa*

Extracts	DIAMETRES D'INHIBITIONS (mm±SD)											
	Gram +				Gram -							Fongal
	SA BAA 977	SA 29213	AV 11563	EF 51229	KP 700603	EC 11775	PA 9027	PA 10145	NG 49226	PM CPC	SE CPC	CA CHUY
PMR HEX	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PMR ACOET	**12,8**±0.0	**13,6**±0.0	0.0±0.0	**12,9**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**11**±0.0	0.0±0.0	**15**±0.0	0.0±0.0
PMR HY-ET	**10,8**±0.0	**9,6**±0.0	**10,3**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**9**±0.0	0.0±0.0	0.0±0.0
PMR HY-ETb	0.0±0.0	**11,1**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**11,6**±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PMR H20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**8,6**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PMEt HEX	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PMEt ACOET	**14,1**±0.0	**13,6**±0.0	0.0±0.0	**14,4**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**8**±0.0	**9,2**±0.0	0.0±0.0	0.0±0.0
PMEt HY-ET	**12,1**±0.0	**12,1**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**7,5**±0.0	0.0±0.0	**9**±0.0	**12,7**±0.0
PMEt HY-ETb	**12,1**±0.0	**12,1**±0.0	**10,1**±0.0	**10**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**10**±0.0	**10,5**±0.0	0.0±0.0
PMEt H20	**8**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**8**±0.0	0.0±0.0	0.0±0.0
PMFr HEX	0.0±0.0	**13,1**±0.0	0.0±0.0	**8,5**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**9,1**±0.0	**16**±0.0	0.0±0.0
PMFr ACOET	0.0±0.0	**10**±0.0	**9,2**±0.0	**8**±0.0	0.0±0.0	**7,4**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**12**±0.0	0.0±0.0
PMFr HY-ET	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PMFr HY-ETb	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**8**±0.0	**8**±0.0	0.0±0.0
PMFr H20	**11,1**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PMFe HY-ETb	**10,2**±0.0	**13,6**±0.0	**11,3**±0.0	0.0±0.0	**9,6**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**9,1**±0.0	0.0±0.0
PMFe H20	0.0±0.0	**12,1**±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	**11**±0.0	**11,9**±0.0
CIPRO 5µg	-	-	-	-	-	-	-	-	-	-	28±0.0	-
GENTA 10 µg	-	-	-	-	-	-	-	-	-	-	**28**±0.0	-
MICO10 µg	-	-	-	-	-	-	-	-	-	-	-	**31**±0.0
TICAR15 µg	-	-	-	-	-	-	-	-	-	**16**±0.0	**28**±0.0	-
AMXCLV 15 µg	-	-	-	-	-	-	-	-	-	**24**±0.0	**21,1**±0.0	-
KETO10 µg	-	-	-	-	-	-	-	-	-	-	-	**30**±0.0

Stem barks extract: PMEt HEX = hexane, PMEt ACOET = ethyl acetate, PMEt HY-ET = hydro-ethanolic, PMEt H20 = Aqueous, PMEt HY-ETb = hydro-ethanolic crude. Fruit: PMFr HEX = hexane, PMFr ACOET = ethyl acetate, PMFr Hy-ET = hydro-ethanolic, PMFr HY-ETb = hydro-ethanolic crude. PMFr H20 = Aqueous. Root: PMR HEX = hexane, PMR ACOET = ethyl acetate, PMR Hy-ET = hydro-ethanolic, PMR H20 = Aqueous, PMR HY-ETb = hydro-ethanolic, Leaves: PMFe H20 = Aqueous, PMFe HY-ETb = hydro-ethanolic crude. Microorganism: SA= *Staphylococcus aureus*, EF= *Enterococcus feacalis*, AV= *Aerococcus viridans*, EC= *Escherichia coli*, KP= *Klebsellia pneumoniae*, CA= *Candida albicans*, SE= *Salmonela enterica*, NG= *Neisseria*

gonorrhea, PA= *Pseudomonas aeruginosa*, PM= *Proteus mirabilis.* Positive control: Cipro= ciprofloxacin, Genta= Gentamicin, Ticar= Ticarcillin, Amxclv= Amoxicillin + Clavulanate acid, Mico= Miconazol, Keto= Ketoconazol

The scavenging power of plants extracts against DPPH has been thought to be due to their hydrogen donating ability. The scavenging potential of extracts of *P. microcarpa* comparable to ascorbic acid and BHT two standards antioxidants as demonstrated in this study suggested that these extracts have compounds with high proton donating ability and could therefore serve as free radical inhibitors.

Concerning the Ferric Reducing Antioxidant Power activity, all the plant extracts interfered with the reduction of Fe^{3+} to Fe^{2+}, suggesting that they possess chelating activity (figure 3).The reducing power was ranging from 0.15 ±0.05 to 2.38 ±0.00DO/µg with the hydro alcohol extract of stem bark exhibiting the best ferric reducing antioxidant power.

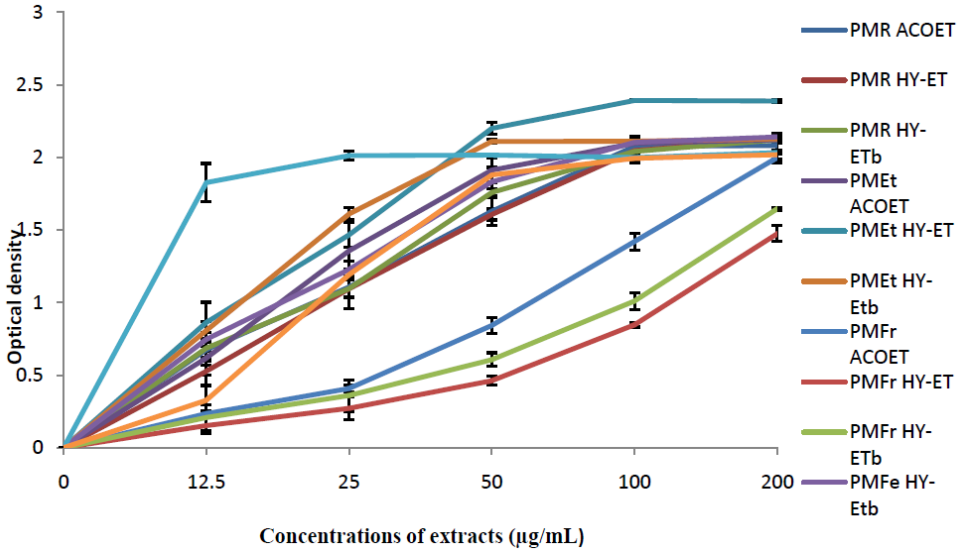

Figure3. *Power reducing activity of the crude extracts of P. microcarpa*

Stem back extract: PMEt ACOET = ethyl acetate, PMEt HY-ET = hydro-ethanolic crude; PMEt H20 = Aqueous, PMEt HY-ETb = hydro-ethanolic crude. Root: PMR ACOET = ethyl acetate, PMR Hy-ET = hydro-éthanolic, PMR HY-ETb = hydro-ethanolic crude. Fruit: PMFr ACOET = ethyl acetate, PMFr Hy-ET = hydro-ethanolic, PMFr H20 = Aqueous, PMFr HY-ETb = hydro-ethanolic crude. Leaves:PMFe HY-ETb = hydro-éthanolic crude. Vit C= Vitamin C, BHT: Butylhydroxytoluene

Concerning the quantitative estimation of the phenolic compounds present in plants extracts, the results are summarize in figure 4 below. From these results, the quantity of phenol compounds is highly variable depending to extracts and plant part. Hydro-ethanol extracts of stem barkis highly riche inphenol compounds (3.594 mgEAG/mg)while the extract from fruits compounds few concentration of phenol compounds.

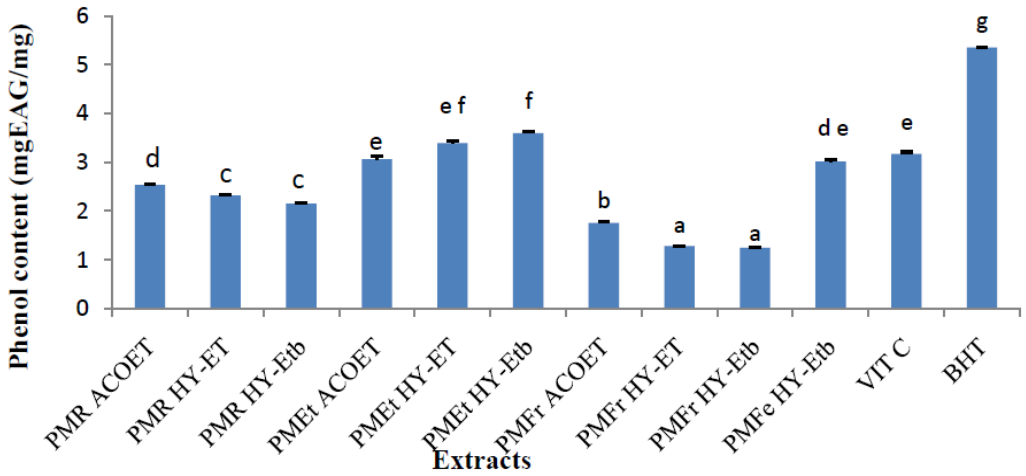

Figure4. Quantity of Phenol content in plants extracts

Stem back extract: PMEt ACOET = ethyl acetate, PMEt HY-ET = hydro-ethanolic crude; PMEt H20 = Aqueous, PMEt HY-ETb = hydro-ethanolic crude. Root: PMR ACOET = ethyl acetate, PMR Hy-ET = hydro-éthanolic, PMR HY-ETb = hydro-ethanolic crude. Fruit: PMFr ACOET = ethyl acetate, PMFr Hy-ET = hydro-ethanolic, PMFr H20 = Aqueous, PMFr HY-ETb = hydro-ethanolic crude. Leaves:PMFe HY-ETb = hydro-éthanolic crude. Vit C= Vitamin C, BHT: Butylhydroxytoluene

3.3. Antimicrobial Activities

The antimicrobial screening of extracts by diffusion method give results in term of inhibition zone diameter ranging from 0-16 mm as shown in (Table3).The significant degree of inhibitory activity of hexane extract of fruit and ethyl acetate extract of roots was observed against *Salmonella enteric* follow by *Enterococcus feacalis 51229* and *Staphylococcus aureus BAA 977*with respective inhibition diameter of16.0 mm and 15.0 mm. The inhibition diameter of ethyl acetate extract of stem bark was between 14.1 mm and 14.4 mm against all gram positives strains.

The MIC determination of selected extracts showed the results range from 156.25 to 2500µg/mL (Table 4). The ethyl acetate extract of stem bark was the most active against *Staphylococcusaureus* 29213 and *Staphylococcus aureus* BAA 977 with MICs values of 156,25 µg/mL and 312,5 µg/mL respectively. The hexane extract of fruit and ethyl acetate extract of roots showed inhibitory activity against *Salmonella enterica* and *Enterococcus feacalis* 51229 with MIC 1250µg/mL.This activity of extract was less than that of standard antibiotic i.e. ciprofloxacin, gentamicin, ketoconazole.

Table4. *MICs, MBCs, MBCs/MICs of different extracts of P. microcarpa*

Extraits/Fractions		Stains/ isolats bacterials			
		SA BAA 977	SA 29213	EF 51229	SE CPC
PMR ACOET	CMI(µg/ml)	625	625	1250	1250
	CMB(µg/ml)	5000	1250	5000	5000
	CMB/CMI (µg/ml)	8	2	4	4
PMET ACOET	CMI(µg/ml)	312,5	156,25	625	Nd
	CMB(µg/ml)	1250	625	2500	Nd
	CMB/CMI µg/ml)	4	4	4	Nd
PMEt HY-ETb	CMI(µg/ml)	625	625	Nd	Nd
	CMB(µg/ml)	5000	2500	Nd	Nd
	CMB/CMI µg/ml)	8	4	Nd	Nd
PMFr HEX	CMI(µg/ml)	Nd	2500	Nd	1250
	CMB(µg/ml)	Nd	1250	Nd	5000
	CMB/CMI µg/ml	Nd	2	Nd	4
PMFr ACOET	CMI(µg/ml)	Nd	1250	Nd	1250
	CMB(µg/ml)	Nd	2500	Nd	10.000
	CMB/CMI µg/ml)	Nd	2	Nd	8
PMFe HY-ETb	CMI(µg/ml)	Nd	625	Nd	Nd
	CMB(µg/ml)	Nd	2500	Nd	Nd
	CMB/CMI µg/ml)	Nd	4	Nd	Nd
Ciprofloxacine	CMI(µg/ml)	Nd	Nd	Nd	0,019
	CMB(µg/ml)	Nd	Nd	Nd	0,039
	CMB/CMI µg/ml)	Nd	Nd	Nd	2

Stem barks extract: PMEt ACOET = ethyl acetate, PMEt HY-ETb = hydro-ethanolic. Root: PMR ACOET = ethyl acetate, Fruit: PMFr HEX = hexane, PMFr ACOET = ethyl acetate. Leaves: PMFe HY-ETb = hydro-ethanolic

These data suggest that the *P. microcarpa* may contain several antioxidants compounds. This wide spectrum of antioxidant activity may be attributed to their secondary metabolites content. In other hand, the high scavenving and the high reducing power of extracts found in this study could be related to their total phenol content [28]. In fact, the principal antioxidant constituents of natural products are phenolic compounds [25]. They are potent free radical terminators [26] and also hydrogen giver to free radicals, and hence, break the reaction of lipid oxidation at the initiation step [1, 27]. Thus, high polyphenolic content will mean a strong antioxidant power and a strong scavenging activity. This is accordance with previous study who present *P. microcarpa* as source of antioxidant compounds [13]. The antimicrobials activities of extracts from different parts of *P. microcarpa* were highly dependent to microorganisms and extracts composition. In fact, the wide range of antimicrobial properties can be explained by the presence of various groups of potentially active secondary metabolites. The

existence of these antimicrobial substances was confirmed by the phytochemical screening which revealed the presence of certain classes of compounds who they antimicrobial activities have already demonstrated. In fact, tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of cell protein synthesis. Tannins prevent bacterial growth by precipitating their proteins [30, 31]. Some flavonoïds have shown several pharmacological activities including antibacterial and antifungal [32]. Many saponins are known to be antimicrobial, to inhibit fungal metabolites in them [33].The antimicrobial activities suggest that the *P. microcarpa* stem barks, fruits, leaves and root may correlates with the observation of previous research which showed that these plants contain substances with antimicrobial activity [24].

4. CONCLUSION

This antimicrobial and antioxidants activities of *P. microcarpa* supports their traditional usage by local communities as the therapeutic agents for the treatment of typhoid fever and another infection disease. Overall, this study has shown that extracts obtained from different parts of *P. microcarpa* could be a potential source of antimicrobial and antioxidant agents. The results achieved suggest further investigation through isolation and biological screening of the potentially active metabolites

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