International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 3 (3), pp. 160-166, October, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antimicrobial and cytotoxicity activities of extract from *Pseudospondias microcarpa* (Anarcadiaceae)

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Accepted 1st September, 2014.

This study was conducted to evaluate the antimicrobial and cytotoxicity activities of extracts from root and stem barks of *Pseudospondias microcarpa.* The root and stem barks were extracted successively by *n*-Hexane, Methylene chloride, ethyl acetate, Methanol, Methanol-Methylene Chloride, and ethanol-water. The antimicrobial activity was evaluated by disc diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Streptomyces viridochromogenes* Tü 57 and Mucor*miehei*. Cytotoxicity activity of extracts was measured by Brine shrimp lethality bioassay and their phytochemical screening was studied.The inhibition zone diameter of the nineteen extracts ranged from 0-15 mm. PMF4 and PMF7 with 15 mm showed moderate activity on *Staphylococcus aureus*. PM11 and PMF6 showed 14 mm on *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* respectively. At 24 h, the percentage of lethality vary from 0-21 % with PMF2 and PM3 having 19 and 21 % respectively. At 32 h, percentage varies from 0-56 % with PMF1, PMF2 and PM3 exhibited 27, 38 and 56 % respectively. Phytochemical screening revealed the presence of sterols, alkaloids, flavonoids, cardiac glycosides, saponins, quinones, glycosides, polyphenols, triterpenes, anthocyanins, anthraquinons, tannins and coumarins. This work revealed the antimicrobial potentials of this plant supporting their traditional use.

Key Words: *Pseudospondias microcarpa,* Anacardiaceae*,* antimicrobial, brine shrimp, phytochemical screening.

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 (Awouafack et al., 2013).In fact, the discovery of antibiotics has decreased the spread and severity of a wide variety of diseases.

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However, the result of their uncontrolled use, the efficiency of many antibiotics is being threatened by the emergence of microbial resistance to existing chemotherapeutic agents (Cowan, 1999). Bacteria and fungi are evolving numerous mechanisms to evade antimicrobial agents and the resistance to old and new antibiotics is increasing (Pareke and Chanda, 2007). The developments of multidrug-resistant (MDR) bacterial strains (Alanis, 2005) have made the search for new and novel antimicrobial substances among the first priorities

within the quest for such materials. In addition the lack of new antifungal agents and the long-term use of antifungal drugs in the treatment of chronic fungal infections have caused the emergence of amphotericin-B and azole resistant *Candida* species (Ficker et al., 2005). Due to these reasons, there is a pressing need to identify new and novel antimicrobial agents that would help in alleviating the problems of emerging resistant bacterial and fungal pathogens.

Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions including infectious diseases (Clardy and Walsh, 2004). Current research on natural products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethno-medicinal uses (Verpoorte et al., 2005). Traditional medicine continues to provide health coverage for over 80% of the world population, especially in the developing world (WHO, 2002)**.** *Pseudospondias microcarpa* is extensively used in herbal medicine in Centre, East, South and Littoral regions of Cameroon in the treatment of various diseases like: Hyperglycemia, gonococci, anemia, diarrhea, filaria, angulillulose, rheumatisms, ankylostomia, ascaridiose, cestodose, malaria, typhoid fever, haemorrhoid, oedemas, rheumatisms, stomach ache, icterus, pediculoses, diabetes, trypanosomiasis, leishmaniasis and elephantiasis (Chenu, 1992;Tanda,1995; Tchoumi,1995; Noumi and Yomi, 2001; Mbita,1999; FAO, 2001). Previous phytochemical and biological investigations showed that the aqueous and methanol-methylene chloride extracts possessed antioxidant activity (Yondo et al., 2009). Petrolum ether extract of leaves showed moderate antibacterial activity on *E. coli* and was found to contain phenolic compounds, tannins and sterols (Kisangau et al., 2007). Ethanolic and dichloromethane extracts of leaves showed moderate antiplasmodial activity (Mbatchi et al., 2006). The present study aimed at evaluating the potential of *Pseudospondias microcarpa* of root bark and stem bark extracts against some Gram-positive, Gram-negative strains of bacteria and fungi, and also their cytotoxicity.

MATERIAL AND METHODS

Plant material

The Stem and Roots barks of *P. microcarpa* were collected in Yaoundé (February 2012) and identified at the Cameroon National Herbarium (HNC) where voucher specimens (41437/HNC) are deposited.

Extraction procedure

The air-dried and powdered root bark (500 g) and stem bark (500 g) of *P. microcarpa* were exhaustively macerated successively with *n*-Hexane (n-Hex, 3 L), Methylene chloride (DCM 3 L), ethyl acetate (AcOEt, 3 L), Methanol (MeOH, 2 L), Methanol-Methylene Chloride (1:1 *v/v*) (MeOH-DCM, 2 L), and ethanol-water (7:3, *v/v*) (EtOH-H_2O, 3 L) respectively at room temperature for 72 h. The macerate was filtered and evaporated under reduced pressure to obtain crude extracts labelled PM8 (10 g); PM9 (7 g), PM10 (40 g), PM6 (33 g), PM7 (35 g), PM12 (70 g) and PM3 (12 g); PM4 (9 g), PM5 (15 g), PM1 (23 g), PM2 (37 g) and PM11 (70 g) respectively.

Part of PM11 (60 g) was solubilized successively with n-Hex, *n*-Hex-AcOEt, (3:1 *v/v*), *n*-Hex-AcOEt, (1:1 *v/v*), AcOEt-MeOH, (3:1 *v/v*), AcOEt, and MeOH followed by filtration to give four main fractions labelled PMF1 (5 g), PMF2 (17 g), PMF3 (4 g), PMF4 (10 g), PMF5 (3 g) and PMF6 (11 g) respectively. The residual was solubilized with water and extract with AcOEt to give PMF7 (2 g).

Phytochemical Screening

The identification of alkaloids, tannin, saponins, flavonoids, glycosids, sterols, triterpens, anthraquinone, phenols, cardiac glycosids, quinones, anthocyanins, coumarins and polyphenols present in the different plant extracts were carried out according to the method of Trease and Evans (2002).

Antimicrobial Assays

Microorganisms

The microorganisms *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Streptomyces viridochromogenes* Tü 57 and Mucor*miehei* were used in this study. *S. aureus* was cultured on pepton medium, *B. subtilis* and *E. coli* in meat extract medium and *M. miehei* and *Str. viridochromogenes* in YMG medium for 12 hours.

Inoculum Preparation

The bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and Streptomyces *viridochromogenes* Tü 57 and the fungus strains *Candida albicans* and *Mucormiehei* were recultivated from fluid nitrogen deposit on agar plates at 37 °C for 12 hours. A loop of each microbial species was removed from the surface of the agar plate and inserted into a test tube half filled with the respective fluid test strain medium and incubated on a round shaker at 37 °C and 180 rounds per minute for 24 hours.

Stock solutions and disc preparation

The different stock solutions of extracts were prepared by

Table 1.Inhibition Zone Diameters (mm).

sample	SA	CA	SV	MM	BS	EC
PM3	0	0	0	0	0	0
PMF2	0	0	0	0	0	0
PMF6	**11**	**12**	0	0	**13**	**11**
PMF4	**15**	0	0	0	**12**	0
PM7	0	0	0	0	0	0
PM12	0	0	0	0	0	0
PM2	0	0	0	0	0	0
PMF5	0	0	0	0	0	0
PMF7	**15**	0	0	0	**15**	**12**
PM11	**13**	**11**	0	0	**14**	**14**
PM9	0	0	0	0	0	0
PM4	0	0	0	0	0	0
PMF3	0	0	0	0	0	0
PM6	0	0	0	0	**11**	0
PM10	0	0	0	0	0	0
PM1	0	0	0	0	0	0
PM8	0	0	0	0	0	0
PM5	0	0	0	**11**	0	0
PMF1	0	0	0	0	0	0
Ciprofluxacin	18	/	12	**/**	25	19
Nystatin	/	14	/	**19**	/	/
DCM / MeOH 1:1	/	/	/	**/**	/	/
DMSO	/	/	/	/	/	/

*SA: Staphylococcus aureus; CA: Candida albicans; SV: Streptomyces viridochromogenes; MM: Mucormiehei; BS: Bacillus subtilis; EC: Escherichia coli.*PM8: *n-*

weighing 10 mg of different extracts and dissolved in 1 ml DCM/MeOH 1:1 and DMSO 10 % respectively for a final concentration of 10 mg/ml. Nystatin and Ciprofluxacin were prepared in the same conditions. 50 µL of each solution was dropped onto sterilized 9 mm diameter paper disks for a final 500 µg / paper disc.

Antimicrobial activity by Discs diffusion assay

In vitro antimicrobial activity was assessed 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 petriplates (90 mm). The plates were allowed to solidify for 5 min and suspensions of test strains diluted 1:4 with the respective fluid test strain. The different loaded discs were placed on the surface of medium and the extracts and compounds were allowed to diffuse for 5 min and the plates were incubated at 37 ^{o}C for 13 hours. Negative control was prepared using respective solvent (DCM/MeOH 1:1/10 % DMSO). Nystatin and Ciprofluxacin were used as positive control. Distinct zone of inhibition were measured with Vernier Calliper and expressed in millimeter (mm). These studies were performed in triplicate (Bauer and Kirby, 1966). The activities of extracts with inhibition zone diameter were classified as follows: 10.5 - 12 mm low activity; 13 – 17 mm moderate activity and > 17 mm high activity.

Cytotoxicity activity by Brine shrimp lethality bioassay

Lethality % of brine shrimps at 100 µg / mL

Brine shrimp lethality bioassay (Meyer et al., 1982) technique was applied for the determination of general toxic property of the root bark and stem bark extracts of *Pseudospondias microcarpa*. Anhydrous *Artemia salina* eggs were suspended in artificial seawater in a 1 L separation funnel by constant aeration and kept under light until hatching depending on the room temperature between one to three days. The aeration was intermitted, unhatched eggs removed from the bottom of the flask and a spot light placed towards one side of the flask to gather brine shrimps which were attracted through chemotaxis. After 2 days of hatching period the eggs were ready for the experiment. More than 20 brine shrimps within 990 µL of artificial seawater were transferred to each well of a 24 - well tissue culture plate. Brine shrimps are observed under the microscope to record dead exemplars. For crude extracts, concentrations of 10 mg / mL in DMSO were prepared, and 10 µL of each sample was dropped into a well containing the seawater. Tests were carried out in triplicate. After 24 and 32 hours, the number of dead brine shrimps was recorded for each well. Plates were kept overnight in the refrigerator to immobilize the brine shrimps for determination of total numbers per well. Lethality in per cent was determined by the formula L = (A-N-B) Z-1 100, with A = number of dead brine shrimps after 24 and 32 hours, N = number of dead brine shrimps before the crude extract was added, B = number of dead brine shrimps in the negative controls (up to 2 % is acceptable), Z = total number of brine shrimps per well, and finally the mean percentage of lethality was calculated.

Table 2. Cytotoxicity assay with *Artemia salin* (%).

Sample	100 µg/mL 24 h	100 µg/mL 32 h	LC_{50} 24 h	LC_{50} 32 h
PM3	**21.00**	**38.00**	/	/
PMF2	**19.00**	**56.00**	**> 100 µg/mL**	**95 µg/mL**
PMF6	0.00	0.00	/	/
PMF4	0.00	3.00	/	/
PM7	0.00	0.00	**/**	**/**
PM12	4.00	4.00	/	/
PM2	0.00	0.00	**/**	**/**
PMF5	4.00	5.00	/	/
PMF7	0.00	0.00	**/**	**/**
PM11	0.00	0.00	/	/
PM9	3.00	3.00	**/**	**/**
PM4	0.00	0.00	/	/
PMF3	0.00	0.00	**/**	**/**
PM6	0.00	2.00	/	/
PM10	0.00	0.00	**/**	**/**
PM1	2.00	4.00	/	/
PM8	0.00	0.00	**/**	**/**
PM5	0.00	0.00	/	/
PMF1	**5.00**	**27.00**	**/**	**/**
Actinomycine D	/	/	< 3.12	no activity
DMSO	**/**	**/**	no activity	no activity

Determination of LC_{50} values

The crude extracts were diluted serially in DMSO, and for each concentration prepared 10 µL was dropped into wells containing 990 µL of artificial seawater containing more than 20 brine shrimps, with concentrations of 100, 50, 25, 12.5, 6.25, and 3.12 µg / mL. Lines were prepared in triplicate. After incubation for 24 and 32 hours, the mean percentage lethality for each sample concentration of each crude extract was plotted against the logarithm of concentrations. The LD_{50} concentration, at which 50 % of the brine shrimps died, was determined from the graph. The highly cytotoxic Actinomycine D and DMSO were used for positive and negative control respectively.

RESULTS

Antimicrobial activity

The inhibition zone diameter ranged from 0-15 mm as shown in table 1. Six extracts (PMF6, PMF4, PMF7, PM11, PM5, and PM6) out of nineteen exhibited inhibition diameters on five strains (*Staphylococcus aureus, Candida albicans, Mucormiehei, Bacillus subtilis and Escherichia coli)*. Five extracts (PMF7, PM11, PMF6, PMF4, PM6) portrayed inhibition zone diameters ranging from 11-15 mm on *Bacillus subtilis,* fourextracts (PMF4, PMF7, PM11, PMF6) on *Staphylococcus aureus* with inhibition zone diameters varying between 11-15 mm, two extracts PMF6, PM11 and three PM11, PMF7, PMF6 with inhibition diameters 11-14 mm on *Candida albicans* and *Escherichia coli* respectively and extract PM5 with diameters of 11 mm on *Mucormiehei.* PMF4 and PMF7 with 15 mm of inhibition diameter showed moderate activity on *Staphylococcus aureus.* PM11 showed with 14 mm on *Bacillus subtilis* and *Escherichia coli.* PMF6 exhibited inhibition with 11 and 14 mm on *Staphylococcus aureus* and *Candida albicans* respectively. Extract PM11 show a broad activity on four microorganisms (*Staphylococcus aureus*, *Candida albicans*, *Bacillus subtilis* and *Escherichia coli)* followed by PMF7 and PMF6 on three microorganisms. In general, extracts and fractions from stem bark and PM11 are more potent than from root bark. No extract exhibited activity on *Streptomyces viridochromogenes* and many extracts showed no activity on all the tested microorganisms. *Bacillus subtilis*is the most sensitive strain followed by *Staphylococcus aureus* and the least sensitive being *Streptomyces viridochromogenes*.

Brine shrimp lethality

The percentage of lethality is shown in table 2. At 24 h, the percentage of lethality vary from 0-21 % with PMF2 and PM3 having 19 and 21 % respectively. Extracts PMF5, PM1 and PMF1 showed very less percentage of lethality at 4, 2 and 5 respectively. Out of nineteen extracts, twelve (PMF6, PMF4, PM7, PM2, PMF7, PM11, PM4, PMF3, PM6, PM10, PM8 and PM5) did not inhibit the growth of *Artemia salina* eggs. At 32 h, percentage varies from 0-56 % with PMF1, PMF2 and PM3 exhibited 27, 38 and 56 % respectively. PM6, PMF4, PM1, PMF5 exhibit less cytotoxicity with 2, 3, 4 and 5 % respectively. After 32 hours, PM3, PMF2, PMF4, PMF5, PM6, PM1 and PMF1 showed an increment of their cytotoxicity on brine shrimp. The number of dead cells increases with extracts PMF1, PMF2 and PM3, at 5.4, 2.94 and 1.80 times respectively. This variation of cytotoxicity of extracts show

Table 3. Preliminary phytochimical study of plants extracts and fraction.

Part of plants	Extracts/ fractions	A	S	F	T	Q	AQ	GC	P	TT	ST	AT	G	L	C
	MeOH	+	+	+	+	+	+	-	+	+	-	+	-	-	+
Roots bark	EtOH-H_2O	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	EtOH-H_2O	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Stem bark	MeOH	+	+	+	+	+	+	-	+	+	+	+	-	-	+
	AcOEt	+	-	+	-	+	+	-	+	+	+	-	-	-	+

A: alcaloids; **S**: saponins; **F**: flavonoids; **T**: tannins; **Q**: quinones; **AQ**: anthraquinons; **GC**: cardiac glycosides; **P**: polyphenols; **TT**: triterpenes; **ST**: sterols; **AT**: anthocyans; **G**: glycosides; **L**: lipids; **C**: coumarines. **MeOH**: methanol; **EtOH-H_2O**: ethanol-eau ; AcOEt : ethyl acetate **+** : present ; **-** : absent.

that their effect on cells is time dependent. PM12 and PM9 did not present any variation in their cytotoxicity after 32 hours meaning that cytotoxicity effect is not time dependent.

The LC_{50} of many extracts were not determined but that of PMF2 was > 100 µg/mL and 95 µg/mL at 24 and 32 h. This result is in accordance with the result of the first experiment thatpresent the cytotoxicity of PMF2 as time dependent.

Phytochemical screening

Phytochemical screening revealed the presence of sterols, alkaloids, flavonoids, cardiac glycoside, saponins, quinones, glycosides, polyphenols, triterpenes, anthocyanins, anthraquinons, tannins and coumarins while lipids were absent table 3.

DISCUSSION

These secondary metabolites obtained exert antimicrobial activity through different mechanisms. Tannins exert its antimicrobial activity by binding with proteins and adhesins, inhibiting enzymes, complexation with the cell wall and metal ions, or disruption of the plasmatic membrane (Cowan, 1999). Moreover, alkaloids have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori, 1994) and interfere with cell division. Saponins have ability to cause leakage of proteins and certain enzymes from the cell (Okwu, 2001). The sensitivity of steroids and the membrane lipids indicate their specific association that causes leakage from liposomes (Majorie, 1999). Flavonoids have the ability to complex with proteins and bacterial cells forming irreversible complexes mainly with nucleophilic amino acids. This complex often leads to inactivation of the protein and loss of its function (Burkill, 1988; Shimada, 2006). Cardiac glycosides are cardioactive compounds belonging to triterpenoïds class of compounds. Their inherent activity resides in the aglycone portions of their sugar attachment (Brian et al., 1985). They exert their hypotensive effect by inhibiting Na+- K+ ATPase. Hence, the presence of these compounds in *Pseudospondias microcarpa* corroborates the antimicrobial activities observed confirming their antimicrobial and cytotoxic properties. The antimicrobial effects of *Pseudospondias microcarpa* extracts against *Staphylococcus aureus*, *Candida albicans*, *Mucormiehei*, *Bacillus subtilis* and *Escherichia coli* suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhoea in man, skin diseases and fungal infections (Rogger et al., 1990). Perekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of *Streptomyces viridochromogenes* which implies that they could not be used for crop protection. The high potency of *Pseudospondias microcarpa* against these bacteria gives scientific basis for its use in folk medicine in the treatment of abscesses, bilious conditions, cough, dysuria, diarrhoeaand candidiasis scurvy (Morton, 1987; Quiroga et al., 2001).

Successive isolation of active compounds from plants depends upon the plant part and type of solvent used in extraction procedure (Parekh and Chanda, 2007). Our results showed that the activity is mainly concentrated in the fractions from ethanol-water extracts of stem bark, indicating that the potential antimicrobial compounds were in the high polarity fractions. Buwa and Staden, (2006) reported that the aqueous extracts were more active against bacteria compared to the ethanol and ethyl acetate extracts. Ethanol-water and water are usually the main solvent used by traditional healers to prepare plant extracts. The fractions derived from ethanol-water extracts produced more consistent antimicrobial activity. These finding support the use of this solvent to prepare extract in traditional medicine to cure infectious diseases. The above bioactivity results serve as a good tool to direct further chemical work towards those extracts with a potential to deliver molecules of medical interest, in a time

effective way.

Brine shrimp lethality is indicative of cytotoxicity, antibacterial activities, pesticidal effects and exert a wide range of pharmacological effects **(**McLaughin et al., 1991). The brine shrimp cytotoxicity assay is also considered as a convenient probe for preliminary assessment of toxicity, detection of fungal toxins, heavy metals, pesticides and cytotoxicity testing of dental materials (Meyer et al., 1982). It can also be extrapolated for cell-line toxicity and antitumor activity (Selvin and Lipton, 2004). It has been observed that LD_{50} values for general cytotoxicity are about one-tenth LD_{50} values in the brine shrimp test (McLaughin et al., 1991). The LD_{50}value of the extract was 95 µg/mL after 32 hours, which indicates that the extract have high pharmacologic actions (Gupta et al., 1996). The presence of saponins, alkaloids and cardiac glycosides may be responsible for the observed brine shrimps lethality activities of the extracts.

Lethality of brine shrimps was determined after 24 hours according to the standard procedure, and in addition after 32 hours to record besides acute cytotoxicity as well chronic toxicity indicated by continued dying of brine shrimps after 24 hours. To check the false positive effect due to lack of food or for other reasons, negative controls were carefully observed to verify that none of the brine shrimps were dead after 32 hours of incubation. The degree of lethality was directly proportional to the concentration of the extract with LC_{50} of PMF2 being > 100 µg/mL and 95 µg/mL at 24 and 32 h respectively. Other crude extracts (PMF6, PMF4, PM8), however, showed no significant differences in percentage mortalities between different concentrations within the same plant species indicating no brine shrimp lethality compared to that of control**.** This significant lethality of several plant extracts to brine shrimp is an indicative of the presence of potent cytotoxic components which warrants further investigation.

CONCLUSION

This work has revealed further potentials of this plant in the area of pharmacology as anticancer and antimicrobial agent supporting the traditional uses of *Pseudospondias microcarpa* in the treatment of various infections. Further studies are required in order to isolate and identify potent bioactive compounds.

LIST OF ABBREVIATIONS

SA: *Staphylococcus aureus*; CA: *Candida albicans*; SV: *Streptomyces viridochromogenes*; MM: *Mucormiehei*; BS: *Bacillus subtilis*; EC: *Escherichia coli.*

PM8: *n*-Hex extract from root bark, PM9: DMC extract from root bark, PM10: AcOEt extract from root bark, PM6: MeOH extract from root bark, PM7: MeOH-DCM (1:1) extract from root bark, PM12: EtOH-H_2O (7:3) (*v/v*) extract from root bark; PM3: *n*-Hex extract from stem bark, PM4: DMC extract from stem bark, PM5: AcOEt extract from stem bark, PM1: MeOH extract from stem bark, PM2: MeOH-DCM extract from stem bark, PM11: EtOH-H_2O (7:3) (*v/v*)extract from stem bark; PMF1: n-Hex fraction from EtOH-H_2O extract of stem bark, PMF2: n-Hex-AcOEt (3:1) fraction, PMF3: n-Hex-AcOEt (1:1) fraction from EtOH-H_2O(7:3) (*v/v*) extract of stem bark, PMF4: n-AcOEt (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF5: AcOEt-MeOH (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF6: MeOH (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF7: AcOEt (aq) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark.

Hex extract from root bark, PM9: DMC extract from root bark, PM10: AcOEt extract from root bark, PM6: MeOH extract from root bark, PM7: MeOH-DCM (1:1) extract from root bark, PM12: EtOH-H_2O (7:3) (*v/v*) extract from root bark; PM3: *n*-Hex extract from stem bark, PM4: DMC extract from stem bark, PM5: AcOEt extract from stem bark, PM1: MeOH extract from stem bark, PM2: MeOH-DCM extract from stem bark, PM11: EtOH-H_2O (7:3) (*v/v*) extract from stem bark; PMF1: n-Hex fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF2: n-Hex-AcOEt (3:1) fraction, PMF3: n-Hex-AcOEt (1:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF4: n-AcOEt (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF5: AcOEt-MeOH (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF6: MeOH (3:1) fraction from EtOH-H_2O extract of stem bark, PMF7: AcOEt(aq) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark.PM8: *n*-Hex extract from root bark, PM9: DMC extract from root bark, PM10: AcOEt extract from root bark, PM6: MeOH extract from root bark, PM7: MeOH-DCM (1:1) extract from root bark, PM12: EtOH-H_2O (7:3) (*v/v*) extract from root bark; PM3: *n*-Hex extract from stem bark, PM4: DMC extract from stem bark, PM5: AcOEt extract from stem bark, PM1: MeOH extract from stem bark, PM2: MeOH-DCM extract from stem bark, PM11: EtOH-H_2O (7:3) (*v/v*) extract from stem bark; PMF1: n-Hex fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF2: n-Hex-AcOEt (3:1) fraction, PMF3: n-Hex-AcOEt (1:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF4: n-AcOEt (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF5: AcOEt-MeOH (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF6: MeOH (3:1) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark, PMF7: AcOEt(aq) fraction from EtOH-H_2O (7:3) (*v/v*) extract of stem bark.

ACKNOWLEDGEMENTS

The authors thank the Cameroon National Herbarium for

collection and identification of *Pseudospondias microcarpa* samples.

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