Current Trends in Biotechnology and Pharmacy
Vol. 12 (2) 128-138 April 2018, ISSN 0973-8916 (Print), 2230-7303 (Online)

Phytochemical screening,antioxidant and antimicrobial efficacy of *Protorhus longifolia*(Bernh. Ex C. krauss) Engl. (Anacardiaceae) seed extracts

Nonsikelolo Yvonne Mhlongo[1], Krishna Suresh Babu Naidu[2], Reddy Kandappa Himakar[1], Sershen[3], Abdelkrim Cheriti[4] and Patrick Govender[1*]

[1.] Dept. of Biochemistry, School of Life Sciences, Westville, University of KwaZulu-Natal, Durban-4000 South Africa. Email:mhlongony@gmail.com

[2.] Department of Biomedical and Clinical Technology, Durban University of Technology, Durban-4000, South Africa. Email: Sureshk@dut.ac.za.

[3.] Dept. of Applied Biology, School of Life Sciences, Westville, University of KwaZulu-Natal, Durban, South Africa. Email: naidoose@ukzn.ac.za

[4.] Phytochemistry & Organic synthesis Laboratory, University of Bechar, 08000, Bechar, Algeria Email: Karimcheriti @ yahoo.com; Phone / Fax (Works): +213 49 81 52 44

*For Correspondence - patrickgovender.ukzn@gmail.com

Abstract

The objective of this study was to elucidate the phytochemical, antioxidant and antimicrobial activities of *Protorhus longifolia* seed extracts in *vitro.* The phytochemical analysis revealed the presence of flavonoids, glycosides and sterols predominately in the methanol and ethanol extracts. Antioxidant activities were evaluated in vitro by DPPH-radical scavenging, H_2O_2 and $ABTS^+$ assays. Methanol extract had the highest DPPH scavenging activity (95% at 200 μg/ml), while hexane extract had the lowest DPPH scavenging activity (16% at 25 μg/ml). Aqueous extract showed high percentage of scavenging activity in $ABTS^+$ radical system compared to other assays. Amongst all the extracts, methanol extract showed significant inhibitory effect against *Staphylococcus aureus* (inhibition zone diameter 16mm) while different seed extracts showed no activity against *Enterococcus faecalis*. While in the case of fungal strains, only methanol extract showed antifungal activity against *Candida albicans* ATCC 10231 (< 9 mm) and *Candida albicans* (< 7 mm). The study indicates that methanol extract of seeds of *P. longifolia* exhibit strong antioxidant and antimicrobial activity and would be potential sources of antioxidant and antimicrobial agents of natural origin and could be used as potential alternative for treating various diseases.

Keywords: Antibacterial; Antifungal; Flavonoids; Methanol extract; Phytochemical; *P. longifolia* seed extract

Introduction

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (1). According to World Health organization (WHO), more than 80% of world's populations of Asia, Africa and Latin America rely on traditional medicine for their primary health care needs (2). Nevertheless, of all the c.250 000 species of higher plants on earth, only a fraction has been examined for all aspects of their potential therapeutic medicinal value (3). The study of plants continues principally for the

discovery of novel secondary metabolites. Around 80% of product were of plant origin and there sales exceeded US $64 billion in 2003

Plants based bioactive molecules that can be used as medicine with wide spectrum of activities can be derived from any part of the plant like leaves, bark, flowers, roots, fruits, seeds etc. (4, 5). This therapeutic property is attributed by variety of chemical substances synthesized by plants as secondary metabolites which include alkaloids, saponins, tannins, flavonoids, glycosides, and anthaquinones (6). Furthermore, the use of traditional medicine and medicinal plants in most developing countries remains more affordable than western medicine and also easily accessible by the poor communities (7). In South Africa in particular, many rural ethnic groups rely on traditional indigenous plant knowledge to treat various diseases in both humans and livestock (8, 9). In the order of 15% of the 24 000 taxa recorded in southern Africa are used in traditional medicines (10) and an estimated 500 plant species are traded in informal medicinal plant markets (11).

Protorhus longifolia (Benrh.) Engl. (Red beech) of Anacardiaceae family is a medium to large mostly dioecious tall tree native to South Africa and Swaziland. The bark of *P. longifolia* has been traditionally used to cure various diseases such as heart water and diarrhea in cows (12), hemiplegic paralysis, heart burn, bleeding from the stomach and in the management of blood clotting related diseases (13). The leaves extracts of *P. longifolia* have been reported to possess antimicrobial activity (14, 15) and 10.2-18% tanning material from the bark (16).

Given the alarming incidence of antibiotic resistance in bacteria of medical significance, and paucity of biological activity from seed of *P longifolia*, the present study was taken to explore phytochemical, antioxidant and antimicrobial activity using different in vitro approaches.

Materials and Methods

Chemicals: Hexane (HE), Ethyl acetate (EA), Chloroform (CH), Methanol (MetOH), Ethanol (EtOH), DPPH (2,2-diphenyl-1-picrylhydrazyl), Hydrogen Peroxide (H_2O_2) and ABTS™ (2,2'-azino-bis) was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used including the solvents, were of analytical grade.

Collection and preparation of plant material: Seeds of *Protorhus longifolia* were collected from Westville campus, University of KwaZulu-Natal (GPS co-ordinates -29.817897, 30.942771). Seeds (100 g) were air-dried and powdered. The dry powder of seeds (100 mg) was individually extracted with hexane (HE), ethyl acetate (EA), chloroform (CH), methanol (MetOH), ethanol (EtOH) and water (H_2O) by the cold percolation method (17). Samples were vigorously mixed using a vortex [Lasec SA (Pty) Ltd.] and left to stand for 24 hours at room temperature. The supernant was collected and allowed to completely evaporate using rotary vacuum evaporator (40°C). The extracts were diluted in 10% DMSO for the bioassay to remove other solvents which would possible affect the testing compounds.

Preliminary phytochemical screening: The preliminary phytochemical screening of aqueous, methanol, ethanol, hexane, ethyl acetate and chloroform seed extracts of *P. longifolia* was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures (18-24).The qualitative phytochemical analysis was performed to identify the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and phenolic compounds from the seed extracts of *P. longifolia*.

In vitro methods

Disc diffusion method: Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer et al. (25) to assess the presence of antibacterial activities of the plant extracts. A bacterial culture (which has been adjusted to 0.5 McFarland standard) was used to lawn Mueller-Hinton agar plates using evenly using a sterile swab. The plates were dried for 15 minutes and then used for sensitivity test. The discs which had been impregnated with series of plant extracts were

placed on Mueller-Hinton agar surface. Each test plate comprises of six discs, one positive control, which is a standard commercial antibiotic disc, one negative control and four treated discs. Sterile filter paper discs (10 mm in diameter, Whatmann) were aseptically transferred on agar surfaces and immediately impregnated with 2 µl (400 µg) of prepared plant extracts and incubated for 24-48 h at 37 °C. Likewise, Vancomycin (30 µg) and Amoxicillin (25 µg) were used as positive control. After the incubation, the plates were examined for inhibition zone. After the incubation, the plates were examined for inhibition zone. The inhibition zone were then measured using calipers and recorded. The test were repeated three times to ensure reliability.

Evaluation of minimum inhibitory concentrations (MICs)

Antibacterial activity: Minimum inhibitory concentration, defined as the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after overnight incubation was determined by monitoring the growth of bacteria in a microplate reader (Synergy HT, BioTek Instruments) at 630 nm by micro dilution method as per NCCLS guidelines (26). The bacterial test cultures used in this study were *Escherichia coli* (ATCC 35218), *Klebsiella pneumoniae* (ATCC 700603), *Staphylococcus aureus* (ATCC 43300), *Enterococcus faecalis* (ATCC 5129) and *Pseudomonas aeruginosa* (ATCC 27853). A serial two fold dilutions of crude extracts were made over the range of 200-1.25 µg/ml to make up in sterile 96-well plates (27). The wells were then inoculated with diluted overnight broth culture initially adjusted to a cell density of $1.5x10^8$ (0.5 McFarland standards) and incubated at 35°C for 24 hours. Neomycin (Sigma) served as a positive control. MIC was recorded as the lowest concentration at which no growth was observed. All experiments were carried out in triplicates.

Antifungal activity*:* MIC values were determined using standard broth microdilution method according to M27-A2 (for yeast) as per CLSI guidelines (28). Briefly, yeast strains *Candida albicans* (ATCC-90028); *Candida krusei* (ATCC-6258); *Candida parapsilosis* (ATCC-22019) were grown aerobically overnight at 35°C on Sabouraud dextrose agar (Merck) plates. Yeasts were harvested and suspended in 1% sterile saline and the turbidity of the supernatants measured spectrophotometrically at 625 nm with an absorbance of 0.08-0.1 equivalents to the No. 0.5 McFarland standard following the NCCLS M27-A2 guidelines. The working suspension was diluted 1:20 in a mixture containing RPMI 1640 medium and 0.165 m morpholine-propanesulfonic acid (MOPS, Sigma-Aldrich) buffered to pH 7.0. The working suspension was further diluted with the medium (1:50) to obtain the final test inoculums (1-5 x 10^3 CFU ml^{-1}). The microtitre plates were allowed to thaw and equilibrate to room temperature under aseptic conditions which contains different concentrations of testing compounds. 100µl aliquots of working inoculum suspensions were dispensed into each well and the plates incubated in an aerobic environment at 35 °C for 24 h. After incubation, 20 µl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-18sulfophenyl)-2H-terazolium salt (MTS, Promega Corporation, Madison, USA) was added directly to each well, incubated at 37 ° C for 4 h and the absorbance recorded at 490 nm on a 96-well plate reader (Mindray MR-96A). Amphotericin- B was used as standard drug for the comparison of antifungal activity. MIC was recorded as the lowest concentration at which no growth was observed.

Study of antioxidant properties

DPPH free radical scavenging activity: The DPPH radical scavenging capacity of different extracts was measured according to Barku et al. (29), with minor modifications. Extract solutions were prepared by dissolving 0.05g of dry extract in 50ml of methanol. An aliquot of 2ml of 0.004% DPPH solution in methanol and 1ml of seed extract in methanol at various concentrations (25, 50, 75, 100 and 200 µg/ml) were mixed and incubated at 25°C for 30 min and absorbance of the test mixture was read at 517 nm using a spectrophotometer (UV min 1240, Shimadzu) against a DPPH control containing only 1 ml of methanol in place of the extract. All experiments were performed in

triplicates and the results were averaged. Ascorbic acid was used as a standard (30). The DPPH radical scavenging activity was calculated by the formula given below

% Radical Scavenging activity = (A_{blank} - A_{sample} / A_{blank}) x 100

Where A_{blank} and A_{sample} represent absorption of the blank and tested extract samples respectively.

H_2O_2 scavenging activity: The H_2O_2 scavenging capacity of different extracts was evaluated according to Ruch et al. (31) with minor modifications. A 4 mM solution of H_2O_2 was prepared in phosphate buffered saline (PBS; pH 7.4) at 20°C. Different extract solutions were prepared 100% methanol and then added to H_2O_2 at a final concentrations (25, 50, 75, 100 and 200 µg/ml). H_2O_2 A_{230} was determined 10 min later using a spectrophotometer (UV min 1240, Shimadzu) against a blank containing PBS without H_2O_2.

The % of inhibition was calculated by the following equation:

% Scavenged [H_2O_2] = (A_C – A_S)/Ac x 100

Where A_C is the absorbance of the control and A_S is the absorbance of the test samples respectively.

$ABTS^+$ radical scavenging activity: The $ABTS^+$ scavenging activities of the seed extracts were determined according to the method of Re et al. (32) with minor modification. Briefly, $ABTS^+$ (2 mM) was prepared by dissolving in 50 ml of phosphate buffered saline (PBS; pH 7.4). $ABTS^+$ was produced by reacting 50 ml of stock solution with 200 µL of 70 mM potassium per sulfate ($K_2S_2O_8$) water solution. The mixture was allowed to stand in the dark at room temperature for 15–16h before use. For the evaluation of antioxidant activity, the $ABTS^+$ solution was diluted with PBS to obtain the absorbency of 0.70 (± 0.02) at 734 nm. Different concentrations (25, 50, 75 and 100µg/ml) of the extracts were prepared and mixed with 1 ml of ABTS solution. The absorbance was read at room temperature after 10 min at 734 nm. PBS solution was used as a blank sample.

The % of inhibition was calculated by the following expression

ABTS scavenging activity (%) = (A_0 – A_1) / A_0 ×100

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the test samples respectively.

Results

Preliminary phytochemical screening: The results of phytochemical screening and qualitative estimation of different extracts of *P. longifolia* studied show that the seeds were rich in flavonoids, glycosides and sterols (Table 1). The seed extracts lacked alkaloids, saponins, phenols and tannins which is worth noting. Particularly, chloroform, methanol and ethanol seed extracts of *P. longifolia* were good sources of different classes of phytocompounds. This indicates that these solvents are effective to isolate active biological compounds due to their high polarity.

Antimicrobial activity: The powdered *P. longifolia* seeds were evaluated for both antibacterial and antifungal activity using hexane, ethyl acetate, chloroform, methanol, ethanol and water using the disc diffusion method in concurrence with the minimum inhibitory concentration (MIC). The results based on the disc diffusion method are presented in Table 2. All crude extracts were found to exhibit antibacterial activity at a concentration of 400 µg/ml, while minimal antifungal activity was observed for different extracts as shown in Table 3. Methanolic and ethanolic extracts displayed high activity against *S. aureus* (>13 mm), *E.coli* (>11 mm), *P. aeruginosa* (>14 mm) and *K. pneumoniae* (>10mm) as presented in Table 2. Antifungal activity was noted maximum foronly methanolic extract against *C. albicans* (<7mm) and *C. albicans* 10321 (<9mm).

The minimum inhibitory concentration (MIC) assay was used to quantify the antibacterial activity of *P. longifolia* seed extracts. An advantage in using the method is that both polar and non-polar compounds can be tested found within the crude extracts. The most potent extract was methanol which had MIC values of 62.5 µg/ml

Table 1. Qualitative phytochemical analysis of various seed extracts of *P. longifolia*

Phytochemical Test	Hexane	Ethyl acetate	Chloroform	Methanol	Ethanol	Water
Alkaloids	–	–	–	–	–	–
Flavonoids	–	–	–	+	+	–
Glycosides	+	–	+	+	+	–
Saponins	–	–	–	–	–	–
Sterols	–	–	+	+	+	–
Phenols	–	–	+	+	+	+
Tannins	–	–	–	–	–	–

Note: "+" indicates the presence and "–"absence.

Table 2. Antibacterial activity of extracts of *Protorhus longifolia* seeds extract by disc diffusion method (400 µg/disc concentration) and zone of inhibition (mm ± SD)

Test organisms	**HE**	**EA**	**CH**	**MetOH**	**EtOH**	**H_2O**	**Neomycin*** (PC)
S. aureus	10 ± 0.47	10 ± 0.47	9 ± 0.47	16 ± 0.47	13 ± 0.47	8 ± 0.94	7
E.coli	7 ± 0.47	7 ± 0.47	7 ± 0.47	13 ± 0.82	11 ± 0.94	8 ± 0.47	15
P. aeruginosa	9 ± 1.41	11 ± 0.82	12 ± 0.47	15 ± 0.47	14 ± 0.47	9 ± 0.47	14
K. pneumoniae	8 ± 0.94	7 ± 0.82	7 ± 0.47	13 ± 0.47	10 ± 0.94	9 ± 0.82	15
E. faecalis	NA	NA	NA	NA	NA	NA	8

HE = Hexane; EA = Ethyl acetate; CH = Chloroform; MetOH = Methanol; EtOH = Ethanol; H_2O = Water; 'NA'=No activity
*PC- Positive control (50 µg/ml)

Table 3. Antifungal activity of extracts of *Protorhus longifolia* seeds by disc diffusion method (400 µg/disc concentration) and zone of inhibition (mm)

Test organisms	**HE**	**EA**	**CH**	**MetOH**	**EtOH**	**H_2O**	**Amphotericin-B* (PC)**
C. albicans	NA	NA	NA	7	NA	NA	12
C. albicans 10231	NA	NA	NA	9	NA	NA	11
C. krusei	NA	NA	NA	NA	NA	NA	9
C. parapsilosis	NA	NA	NA	NA	NA	NA	10

HE = Hexane; EA = Ethyl acetate; CH = Chloroform; MetOH = Methanol; EtOH = Ethanol; H_2O = Water; 'NA 'indicates = no activity; *PC = Positive control (20 µg/ml)

against *P. aeruginosa* and was the same as the positive control (Table 4). This antibacterial effect could have resulted from the bioactive composition of phytochemicals present in the plant. Phytochemicals are known to exhibit antimicrobial activity through different mechanisms (33). Flavonoids disrupt microbial cell wall by forming complex with extracellular soluble proteins in the bacteria (34,35). Predominately, flavonoid rich plants possess a broad spectrum of antimicrobial activity (34)

Antioxidant properties: The free radical scavenging activity of *P. longifolia* was studied by its ability to reduce the DPPH, a stable free radical. The DPPH inhibition of various seed extracts are shown in Fig. 1. Percentage scavenging activity of different solvent extracts increased with increase in concentrations. Methanol, ethanol and water extracts exhibited more effective scavenging activity than other solvent extracts. Methanol extract had the highest DPPH scavenging activity (95% at 200 µg/ml), while hexane extract had the lowest DPPH scavenging activity (16% at 25 µg/ml) as compared to the standard control (96% at 200 µg/ml). The reduction in the number of DPPH molecule can be correlated with the available number of hydroxyl groups. Hence the significant scavenging activity may be due to the presence of hydroxyl groups present in the extracts (35).

In the H_2O_2 scavenging assay, all crude extracts quenched the H_2O_2 radical at different concentrations (Fig. 2). Water extract had the highest H_2O_2 scavenging ability (90% at 200 µg/ml), where we can consider it nearly fully inhibiting, while hexane extract had the lowest H_2O_2 scavenging activity (22% at 25 µg/ml). Water extract was more effective as it exhibited the same activity as the standard control (90% at 200 µg/ml). However, the reducing power of various extracts increased with increasing dose.

In ABTS assay, aqueous extract had the highest $ABTS^+$ scavenging activity (96% at 200 µg/ml), while hexane had the lowest $ABTS^+$ scavenging activity (17% at 25 µg/ml) as shown in Fig. 3. This wide range of antioxidant activity may be attributed to the wide variety of bioactive compounds like phenolics, flavonoids, tannins etc. present in the plant. In all antioxidant assays evaluated, the extracts showed efficient activity as compared to standard ascorbic acid.

Discussion

Ethno-pharmaceutical studies and alternative medicine have become increasingly valuable in the recent years and medicinal plants

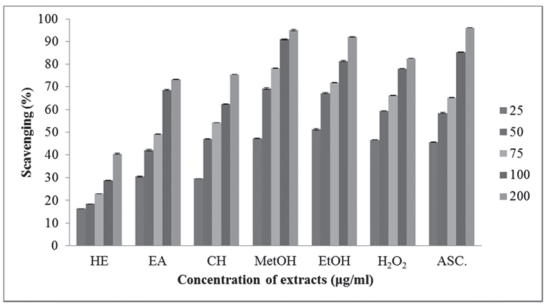

Fig. 1. DPPH radical scavenging activity of different concentrations of *P. longifolia* seed extracts and ascorbic acid.

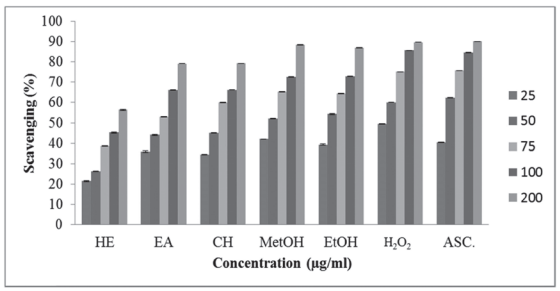

Fig. 2. H_2O_2 scavenging activity of different concentrations of crude extracts and ascorbic acid.

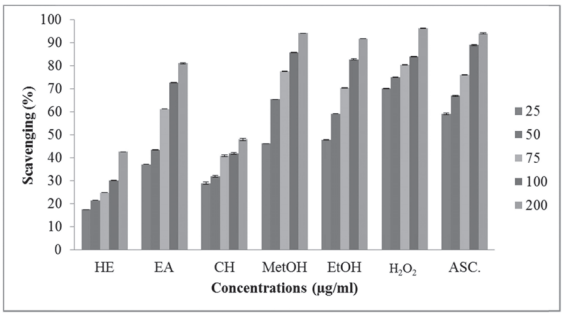

Fig. 3. ABTS+ scavenging activity of different concentrations of crude extracts and ascorbic acid

Table 4. In vitro antibacterial activity of *P. longifolia* extracts (MIC in µg/ml)

Test organisms	HE	EA	CH	MetOH	EtOH	H_2O	PC*
S. aureus	200	200	200	100	200	200	128
E. coli	200	100	-	150	100	200	62.5
P. aeruginosa	150	100	150	62.5	100	150	62.5
K. pneumoniae	150	100	200	100	>100	150	62.5
E. faecalis	NT	NT	NT	NT	NT	NT	100

S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; P.aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; E. faecalis: Enterococcus faecalis.
HE = Hexane; EA = Ethyl acetate; CH = Chloroform; MetOH = Methanol; EtOH = Ethanol; H_2O = Water; '- 'indicates = no activity
*PC = Positive Control (Neomycin) - 20 µg/ml and NT=Not tested.

are now part of the health care system. The medicinal plant conservation programs and their sustained supply are part of global health strategy (36).

The present findings of phytochemical content were in agreement with another study carried out by other researchers Moosa et al. (37, 38) who carried out in vitro evaluation of antihyperlipidemic potential of triterpenes from stem bark of *Protorhus longifolia* commonly used by Zulu traditional healers to manage blood clotting related diseases. Presence of flavonoids, glycosides and phenols in methanolic extract may be responsible for its free radical scavenging activity. It is not surprising that there are differences in the antimicrobial effects of plant groups, due to phytochemical properties and differences among species. The investigated plant seeds did not show presence of alkaloids; however, negative results do not mean absence of bioactive constituents. Active compound may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed (39, 40). Lack of activity can thus only be proven by using large doses (41). Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents (42).

The methanolic extract of seeds of *P. longifolia* displayed highest antibacterial activity against the majority of pathogens tested. This indicates that the extracts possess substances such as flavonoids, glycosides, and tannins which have been linked with the healing properties of plants (43). The relatively high antibacterial activity of methanol extract and ethanol extract could imply that the methanol and ethanol solvents extracted more bioactive compounds and accordingly contributed to a great degree of inhibition. Water is mostly used by traditional healers in preparing extracts however, plant extracts derived from methanol and ethanol have also been found to be active against bacteria from this study. *S. aureus* (Gram positive) was more susceptible to the extracts than Gram negative pathogens tested as shown in Table 4. Antimicrobial studies have shown that Gram negative bacteria show a higher resistance to plant extracts than Gram positive bacteria (44).This may be as a result of the variation in the cell wall structure of Gram positive and Gram negative. More especially, Gram negative bacteria have an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (45). Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. It is thought that observed

differences may result from the doses used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains (46).

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (47). The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. We can infer that, the high antioxidant activities of the methanolic, ethanolic and water extracts may be probably due to the presence of compounds with hydroxyl group.

Hydrogen peroxide is a weak oxidizing agent that has an ability to directly inactivate enzymes by oxidation of essential thiol (-SH) groups (48). It can cross cell membranes rapidly, and once inside the cell, H_2O_2 probably reacts with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical which may be the origin of many of its toxic effects (49). It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The methanol extract, chloroform extract, water extract and ethanol extract scavenged H_2O_2 with increasing concentrations and this may be attributed to the presence of phenolics, which could donate electrons thereby neutralizing it into water.

$ABTS^+$ assay can be used as a guide that reflects the antioxidant activity of different extracts. ABTS is a fairly stable free radical which involves the generation of $ABTS^+$ mono-cation without involvement of intermediary cation. High antioxidant activity of *P. longifolia* seeds maybe due to bioactive compounds present in the plant extracts such as flavonoids, which was evident in both the methanolic and ethanolic extracts. Flavonoids are responsible for the antioxidant properties of the medicinal plants. Biological effects of flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation. Furthermore, flavonoids act as scavengers of free radicals such as reactive oxygen species (ROS), and also prevent their formation by chelating metals. Furthermore, flavonoids contain broad spectrum of biological activity like antimicrobial properties (50, 51).

Conclusion

The seed extracts of *P. longifolia* is a potentially a good source of antimicrobial agent and demonstrate the importance in medicine and in assisting primary health care in this part of the world. Preliminary phytochemical analysis during the present study also ascertains the presence of some potential group of bioactive substances, but the nature of active phytochemical responsible for antibacterial activity cannot be ascertained. Amongst various solvents investigated to access the extraction effectiveness, methanol extract showed remarkable activity. Further studies are necessary to isolate and characterize the active components of the extracts and also to elucidate their antimicrobial mechanisms of action and to further validate pharmacological evaluation.

Acknowledgements:

This work was financially supported by the National Research Foundation (NRF) of South Africa.

Conflict of Interest: Authors declare no conflict of interest.

References:

1. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M and Yoga Latha L. (2011). Extraction, Isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary, and Alternative Medicines* 8:1-10
2. AFRO, W. (2010). African Traditional Medicine day. *The African Health Monitor* 31 August Special Issue
3. Wagner, H., Hikino, H. and Farnsworth NR. (2012). *Economic and Medicinal Plant Research*. Academic Press.
4. Nwankwo, I., Ukaegbu-Obi, K., Mushtaq, A., Pervez, S. and Hussain S, et al. (2014).

Preliminary phytochemical screening and antibacterial activity of two Nigerian medicinal plants (Ficus asperifolia and Terminalis catappa). *Journal of Medicinal Plant and Herbal Therapy Research* 2: 1-5

5. Parekh, J. and Chanda S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research* 10:175-181

6. Rios, J. and Recio M. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology* 100:80-4

7. Hoareau, L. and DaSilva E. J. (1999). Medicinal plants: a re-emerging health aid. *Electronic Journal of Biotechnology* 2:3-4

8. Masika, P. and Afolayan A. (2002). Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. *Journal of Ethnopharmacology* 83:129-34

9. McGaw, L. and Eloff J. (2008). Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *Journal of Ethnopharmacology* 119:559-74

10. Van Wyk, B. E. (2008). A broad review of commercially important southern African medicinal plants. *Journal of Ethnopharmacology* 119:342-55

11. McGaw, L., Jäger, A., Grace, O., Fennel, C. and Van Staden J. (2005). Medicinal plants. In *Ethics in Agriculture—An African Perspective*:67-83: Springer. Number of 67-83 pp.

12. Dold, A.P.C. M.L. (2001). Traditional veternary medicine in the Alice district of the Eastern Cape Province, South Africa. *South African Journal of Science* 97:375-9

13. Moosa Lazarus, G.G., Gwala, P.E., Oyedeji, A.O. and Opoku A.R. (2011). In Vitro Anti-platelet Aggregation, Antioxidant and Cytotoxic Activity of Extracts of Some Zulu Medicinal Plants. Journal of Natural Products 4: 136-146

14. Suleiman, M.M., Bagla, V., Naidoo, V. and Eloff J. N. (2010). Evaluation of selected South African plant species for antioxidant, antiplatelet, and cytotoxic activity. *Pharmaceutical Biology* 48:643-50

15. Suleimana, M.M., McGaw, L.J., Naidoo, V. and Eloff J.N. (2010). Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *African Journal of Traditional, Complementary and Alternative Medicines : AJTCAM / African Networks on Ethnomedicines* 7:64-78

16. Mosa, R.A., Naidoo, J.J., Nkomo, F.S., Mazibuko, S.E., Muller, C.J. and Opoku A. R. (2014). In vitro antihyperlipidemic potential of triterpenes from stem bark of *Protorhus longifolia*. *Planta Medica* 80:1685-91

17. Chanda, S., Dudhatra, S. and Kaneria M. (2010). Antioxidative and antibacterial effects of seeds and fruit rind of nutraceutical plants belonging to the Fabaceae family. *Food & Function* 1:308-15

18. Mallikharjuna, P., Rajanna, L., Seetharam, Y. and Sharanabasappa, G. (2007). Phytochemical studies of *Strychnos potatorum* L.f.-A medicinal plant. *Journal of Chemistry* 4:510-8

19. Savithramma, N., Rao, M.L. and Suhrulatha, D. (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research* 8:579-84

20. Farnsworth, N. R. (1966). Biological and phytochemical screening of plants. *Journal of Pharmaceutical Sciences* 55:225-76

21. Kumar, A., Ilavarasan, R., Jayachandran, T., Decaraman, M. and Aravindhan, P. et al. (2009). Phytochemicals investigation on a tropical plant, Syzygium cumini from Kattuppalayam, Erode district, Tamil Nadu,

South India. *Pakistan Journal of Nutrition* 8:83-5

22. Treare, G. and Evans, W. Pharmacognosy 17th edn, Bahive Tinal, London, 1985: 149
23. Thite, S., Chavan, Y., Aparadh, V. and Kore, B. (2013). Preliminary phytochemical screening of some medicinal plants. *International Journal of Pharmaceutical, Chemical and Biological Sciences* 3: 87-90
24. Paris, R. and Moyse, H. (1969). Precis de matiere medicinale. Paris: Masson,1969.
25. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45:493-6
26. Villanova, P. (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, which Grows Aerobically. *NCCLS*:5th ed., Approved Standard M7-A5
27. Annamalai, A., Christina, V.L.P., Sudha, D., Kalpana, M and Lakshmi, P.T.V. (2013). Green synthesis, characterization and antimicrobial activity of AuNPs using *Euphorbia hirta* L. leaf extract. *Colloids and Surfaces B-Biointerfaces* 108:60-5
28. NCCLS. (2002). Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard M27-A2. Villanova, PA,: CLSI Document. Clinical and Laboratory Standards Institute
29. Barku, V.Y.A., Boahen, Y.O., Ansah, E.O., Dayie, N.T.K.D. and Mansah, F.E. (2013). In-Vitro assessment of antioxidant and antimicrobial activities of methanol extracts of six wound healing medicinal plants. *Journal of Natural Sciences Research* 3:74-80
30. Ramnik, S., Narinder, S., Saini, B. and Rao, H.S. (2008). In vitro antioxidant activity of pet ether extract of black pepper. *Indian Journal of Pharmacology* 40:147
31. Ruch, R.J., Cheng, S.J., Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 10:1003-8
32. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26:1231-7
33. Owoyele, B.V., Oyewole, A.L., Alimi, M.L., Sanni, S.A. and Oyeleke, S.A.(2015). Anti-inflammatory and antipyretic properties of *Corchorus olitorius* aqueous root extract in Wistar rats. *Journal of Basic and Clinical Physiology and Pharmacology* 26: 363-368
34. Pourcel, L., Routaboul, J.M., Cheynier, V., Lepiniec, L. and Debeaujon, I. (2007). Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends in Plant Science* 12:29-36
35. Singh, G. and Kumar, P. (2014). Antibacterial activity of flavonoids of *Withania somnifera* L. *International Journal of Green Pharmacy* 8:114-118.
36. Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Kähkönen. M. et al. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology* 56:3-12
37. Goveas, S.W. and Abraham, A. (2013). Evaluation of antimicrobial and antioxidant activity of stem and leaf extracts of *Coscinium fenestratum*. *Asian Journal of Pharmaceutical and Clinical Research* 6
38. Ibrahim, M.M., Al Sahli, A.A.A., Alaraidh, I.A., Al-Homaidan, A.A., Mostafa, E.M. and El-Gaaly, G.A. (2015). Assessment of antioxidant activities in roots of Miswak (Salvadora persica) plants grown at two

different locations in Saudi Arabia. *Saudi Journal of Biological Sciences* 22:168-75

39. Taylor, J., Rabe, T., McGaw, L., Jäger, A. and Van Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. *Plant Growth Regulation* 34:23-37

40. Farnsworth, N.R. (1993). Ethnopharmacology and future drug development: the North American experience. *Journal of Ethnopharmacology* 38:137-43

41. Wall, M., Wani, M., Brown, D., Fullas, F., Olwald J. et al. (1996). Effect of tannins on screening of plant extracts for enzyme inhibitory activity and techniques for their removal. *Phytomedicine* 3:281-5

42. Lindsey. K., Jäger, A.K., Raidoo, D.M. and Van Staden, J. (1998). Screening of plants used by Southern African traditional healers in the treatment of dysmenorrhoea for prostaglandin-synthesis inhibitors and uterine relaxing activity. *Journal of Ethnopharmacology* 64:9-14

43. Whitehead, S.R., Jeffrey, C.S., Leonard, M.D., Dodson, C.D., Dyer, L.A. and Bowers, M.D. (2013). Patterns of secondary metabolite allocation to fruits and seeds in *Piper reticulatum*. *Journal of Chemical Ecology* 39:1373-84

44. Palombo, E.A. and Semple, S.J. (2001). Antibacterial activity of traditional Australian medicinal plants. *Journal of Ethnopharmacology* 77:151-7

45. Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R. andPopov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *Journal of Ethnopharmacology* 64:235-40

46. Çetin, E and Gürler, N. 1989. Antibiotic susceptibility tests of bacteria. *KükemDergisi* 2:97-105

47. Soares, A.A., de Souza, C.G.M., Daniel, F.M., Ferrari, G.P, da Costa, S.M.G. and Peralta, R.M. (2009). Antioxidant activity and total phenolic content of *Agaricus brasiliensis* (Agaricus blazei Murril) in two stages of maturity. *Food Chemistry* 112:775-81

48. Bhaskar, H. and Balakrishnan, N. (2009). In vitro antioxidant property of Laticiferous plant species from western ghats Tamilnadu, India. *International Journal of Health Research* 2:163-170

49. Miller, M., Sadowska-Krowicka, H., Chotinaruemol, S., Kakkis, J.L. and Clark, D.A. (1993). Amelioration of chronic ileitis by nitric oxide synthase inhibition. *Journal of Pharmacology and Experimental Therapeutics* 264:11-6

50. Williams, R.J., Spencer, J.P. and Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radical Biology and Medicine* 36:838-49

51. Heim, K.E., Tagliaferro, A.R. and Bobilya, D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry* 13:572-8