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Phytochemical screening, thin-layer chromatography and antimicrobial activity study of *Parquetina nigrescens* leaf extracts

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Abstract. *Parquetina nigrescens* leaves have been used in traditional medicine as an important and highly efficacious herbal remedy and have been recommended as a potential source of antimicrobial agent**.** Three extracts of the plant obtained using *n*-hexane, methanol and water were used as solvents. Phytochemical analysis of the plant extracts showed important bioactive compounds such as flavonoids, saponins, tannins, terpenes, steroids, phenols and glycosides, but alkaloids were absent in all the three extracts. Agar disk diffusion method was used to study the antimicrobial activity of the extracts at different concentrations which showed activity against three gram negative bacteria *Escherichia coli, Klebsiella pneumoniae* and *Proteus spp.*, one gram positive bacteria *Staphylococcus aureus,* and two fungus *Aspergillus flavus* and *Candida albicans* with zone of inhibition ranging from 5-15 mm for *n*-hexane extract, 6-16 mm for methanol extract and 1-11 mm for aqueous extract compared to zone of inhibition for the standard antibacterial drug, 0.5 mg/mL Streptomycin that ranges from 13 – 37 mm and the zone of inhibition for the standard antifungal drug 5 mg/mL fluconazole that ranges from 24 – 25 mm. *Klebsiella pneumoniae* was the most inhibited while *E. coli* was the least inhibited by the extract, and *Candida albicans* was found to be resistant to the extracts in all the concentrations. TLC finger-printing of the extracts using the solvent system – butanol : acetic acid : ethanol : distilled water in the ratio 50:10:10:30 showed spots with peaks different retention times ranging from 0.24 - 0.74 cm. The results provide justification for the use of the plants in folk medicine to treat various infectious diseases.

Keywords: TLC finger-print; antimicrobial agent; phytochemicals; *Parquetina nigrescens* extracts.

1. Introduction

Plant extracts with medicinal value have been used to treat many diseases that can either be due to bacterial, fungal or parasitic infections among many others. Plants with medicinal value produce certain chemical substances known as phytochemicals that have antimicrobial activity [1]. *Parquetina nigrescens* known as “ewe ogbo” in Yoruba language in Nigeria, the study plant under investigation is claimed by some folk medicine practitioners to be effective against infertility in males and also used in the treatment of diseases such as hypertension, kidney problems, severe constipation, venereal diseases, stomach ulcers, dysentery, gonorrhea, diarrhea, cardio toxicity, GIT disorders, diabetes, fatigue, menstrual disorder, etc. [2].

As already known, there is a growing interest by researchers to investigate the antimicrobial activity of medicinal plants for new antimicrobial agents since the acceptance of traditional medicine as alternative form of health care [3]. Also, the alarming incidence of antibiotic resistance by microorganism necessitated the development of new and effective therapeutic agents from plants extracts and their screening for phytochemicals that could serve as alternative antimicrobial drugs for treatment of infectious disease ravaging lives nowadays. *P. nigrescens* is claimed by folk medicine to be effective against infertility in males, aside its other medicinal uses [4]. Some previous work done on the plant included evaluation for antioxidant activities in terms of scavenging effects, reducing power and inhibition, and was observed to scavenged 2,2-diphenyl-1-picrylhydrazyl (DPPH) generated radicals in the increasing order of flavonoid > methanol > aqueous at 100 mg/mL. Also, the reducing power was observed to follow the same order as in above, while its extracts at 50 mg/mL exhibited significant (at $p < 0.05$) inhibitory effects on Fe^{2+}/ascorbate - induced lipid peroxidation in rat liver mitochondria [5]. *Parquetina nigrescens* was also investigated as economic plant grown in West Africa for antimicrobial properties, anti-sickling and toxicological profiling, etc.

2. Experimental

This study was carried out in the Department of Chemistry of the Benue State University and the materials and method used for this study are as described hereafter.

2.1. Preparation of sample

The leaves of *Parquetina nigrescens* were collected from Ebonda-Ukpa in Oju Local Government Area of Benue State, Nigeria in November 2017 and was identified by Mr. Waya Joshua, a botanist in the

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Department of Biological Sciences, Benue State University, Makurdi, Nigeria. The leaves were then washed with distilled water, air-dried at low temperature for 3 weeks, and later, crushed using a mortar and pestle, sieved then stored in a cool and dry place for further use.

2.2. Extract preparation

The powdered leaves (100 g) were macerated in a clean container with 400 mL of *n*-hexane (from Sigma-Aldrich) for 72 h and kept at room temperature and shook occasionally after which it was filtered. The filtrate was concentrated under pressure using the rotary evaporator at 60 ^oC and was later air dried at room temperature to obtain the extract. The dried sample was weighed, and the yield was recorded. The residue was macerated again with methanol (from VWR International) and distilled water successively based on increasing polarities. The extracts were then used for phytochemical screening and TLC analysis [6].

$$\%\ \text{Yield of extract} = \frac{Amount\ of\ extract}{Amount\ of\ sample\ taken}\ x\ 100$$

Weight of sample taken = 100 g.

2.3. Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as in powder specimens using the standard procedures [7].

2.4. Thin Layer Chromatography (TLC) of the extracts

Thin layer chromatography was done on the three extract of *Parquetina nigrescens* using a glass TLC plate whose surface was coated with silica gel.

A lead pencil was used to mark the TLC plates. A light pencil line was drawn across the plate about 1 cm from the lower edge of the end of the TLC plates. This is the *origin*: the line on which the TLC plate was spotted. Under the line, the name of the samples were marked lightly and a distance of about 0.5 to 1 cm was left between the spotted samples so that they do not mix or overlap together while separating. A capillary tube was used for spotting the extracts which were first dissolved in 1 ml of its respective macerating solvent. As a rule of thumb, a concentration of 1% usually works well for TLC analysis. If the sample is too concentrated, it will run as a smear or streak. After filling the capillary by dipping it in the various extracts of *Parquetina nigrescens*, it was quickly touched on the cotton wool, so the spot is not larger than 1 to 2 mm diameter (the smaller the spot the better the TLC separation). The spotted TLC plates were developed in a beaker with a watch glass on the top (used as TLC tank), ensuring that the tank was cleaned and dried. The ratios, 50:10:10:30 of butanol : acetic acid : ethanol : distilled water was measured, and the developing solvent was poured into the beaker (developing chamber) to a depth of about ½ cm and shaken. To aid in the saturation of the TLC chamber with solvent vapors, part of the inside of the beaker was lined with filter paper. The beaker was covered with a watch glass, swirled gently, and allowed to stand before use. The forceps was used to carefully place the TLC plate in the developing beaker and covered with the watch glass and leaned against the wall of the beaker. It was ensured that the spots were not below the solvent level to avoid being washed into the solvent. The solvent rose up the TLC plate by capillary action.

After the solvent has risen to the top of the plate, within 1 cm from the top, the plate was removed with forceps and the solvent front was marked immediately with a pencil. The solvent was allowed to completely evaporate from the plate and visualized. The plate was first examined in daylight, the spots were outlined with a pencil. After it was viewed under the UV light and finally put in an iodine vapor tank. After sitting inside the tank for a few minutes, the compound spots turn brown and the spots were marked with a pencil after development in iodine vapor because the iodine color fades with time [8].

The Rf value was calculated for all the spots observed on the TLC plate and the components in the unknown was compared with the Rf data for known compounds, and it was calculated using the expression below:

$$\text{Rf} = \frac{distance\ spots\ travelled}{distance\ solvent\ front\ travelled}$$

Rf value essentially describes the distance travelled by the individual component.

2.5. Antimicrobial activity

Test organisms. The bacterial isolates used in this study are *Staphylococcus aureus, Klebsiella pneumoniae, Proteus spp*, *Escherichia coli*, and fungal isolates used are *Aspergillus flavus* and *Candida albicans*, obtained from Biology Department, Benus State University, Nigeria. Each bacterial solution was standardized at 1.5 x 10^8 cfu/mL.

Samples of 20 g, 40 g, and 60 g of the powdered leaves were measured each into an air tight glass bottle, and 100 mL of *n*-hexane, methanol and distilled water respectively were macerated successively based on increasing polarities to get a concentration of 200 mg/mL, 400 mg/mL, 600 mg/mL.

Preparation of filter paper disc. Discs of 0.5 mm were prepared from Whattman No. 1 filter paper. This was done by punching the filter papers using a paper punch. The discs were washed and placed in glass Petri dishes and hot air oven to sterilize and remove moisture [9]. The discs used for the antimicrobial activity were impregnated with formulated stock solution of *Parquetina nigrescens* extracts (*n*-hexane, methanol and aqueous) at 200 mg/mL, 400 mg/mL and 600 mg/mL concentration and left to stand for 5 min to allow filter papers to absorb the extract. The discs were also impregnated with the controls. Aseptic techniques were ensured to maintain sterility for effectiveness and accuracy in results without contamination. The forceps used for picking the discs was first sterilized using a spirit lamp and left to cool. The forceps were sterilized after every pick [10].

Preparation of media. The media used were Nutrient agar and Potato dextrose agar (PDA), which were prepared according to commercially given instructions. The media was autoclaved for 15 min at 121 ^oC, after which, it was left to cool down to room temperature and

poured into sterilized Petri dishes in a laminar flow to give uniform depth of 3-4 mm, covered and allowed to solidify at 45 °C. The media was turned upside down to avoid condensation of moisture on the surface of the media.

2.6. Antibacterial susceptibility test

Antibacterial activity was determined using agar disc diffusion method [11]. In this technique, the bacteria were first grown on nutrient agar at 37 ºC for 24 h before use. The isolates were later subculture into nutrient agar using sterile wire loop by streak method. The nutrient agar medium was used and prepared according to the formulation, sterilized and poured into a sterile plate to a depth of 4 mm. The antibacterial activities of leaves of *Parquetina nigrescens* were tested against the organisms such as gram (-) *Escherichia coli, Proteus spp,* and *Klebsiella pneumoniae*, and gram (+) such as *Staphylococcus aureus.* After 24 h of incubation, the discs were then immersed with the solution of the extract at different concentrations (200 mg/mL, 400 mg/mL and 600 mg/mL). The plates were allowed to stand on the laboratory for 1 h to allow proper inflow of the solution into the medium before incubating the plate in the incubator at 37 ºC for 24 h.

The diameter of the zone of inhibition was measured. The effect of the extracts on bacteria isolates were compared with standard drug Streptomycin at concentration of 0.5 mg/mL as treatment while *n*-hexane, methanol and distilled water were used as control and the experiments repeated two times.

2.7. Antifungal susceptibility test

Antifungal activity was determined using Poisoned food technique method [12]. 2 mL of the extract concentration were dispensed in Petri dishes after which 15-20 mL of molten PDA was added. The PDA agar-extract mixture was swirled gently on the work bench to ensure even dispersion of the extracts and allowed to solidify. 4 mm diameter of mycelia obtained from the edge of a five-day old culture of each test fungi was inoculated centrally into the medium. Two replicates were used for each fungal isolate. Negative controls were Petri plates with the organism with no botanical extract and treatment, fluconazole at concentration of 5 mg/mL. The Petri dishes were arranged in complete randomized design and incubated at 27-30 ºC for 5-7 days. Inhibition for fungal growth was calculated using the formula:

$$\text{Growth inhibition} = \frac{L1-L2}{L1} \text{x } 100,$$

where L_1 - growth of the pathogen in control and L_2 - growth of the pathogen with treatment

3. Results and discussion

Phytochemical screening result of the extracts in Table 1 showed the presence of terpenes, glycosides, steroids, phenols, tannins and flavonoids in *n*-hexane extract, except saponins and alkaloids.

Table 1. Phytochemical screening of the extracts

Phytochemicals	*n*-Hexane	MeOH	Aqueous
Phenols	+	+	+
Saponins	-	+	+
Terpenes	+	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Steroids	+	+	+
Tannins	+	+	+
Alkaloids	-	-	+

Key: + = present; - = absent.

In the methanol and aqueous extracts, the presence of saponins, flavonoids, steroids, tannins, glycosides, terpenes and phenols were found while alkaloids were absent in the extracts. Alkaloids were absent in all the three extracts. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. This study agrees with phytochemical investigations done on same plant by other authors [13, 14], but differ only in the absence of alkaloids, which could be due to differences in geographical and ecological factors.

Table 2. TLC finger print

Daylight visualization	Rf (cm)	Spot color
Spot 1 (*n*-hexane)	0.37	Light green
Spot 2 (*n*-hexane)	0.72	Army green
Spot 3 (methanol)	0.37	Brown
Spot 4 (methanol)	0.63	Light brown
Spot 5 (methanol)	0.72	Army green
Spot 6 (aqueous)	0.35	Brown
Spot 7 (aqueous)	0.41	Brown
Spot 8 (aqueous)	0.72	Yellow
Under UV 254 nm		
Spot 1 (*n*-hexane)	0.35	Green
Spot 2 (*n*-hexane)	0.24	Green
Spot 3 (*n*-hexane)	0.63	Green
Spot 4 (methanol)	0.36	Green
Spot 5 (methanol)	0.52	Green
Spot 6 (aqueous)	0.26	Green
Spot 7 (aqueous)	0.53	Green
Spot 8 (aqueous)	0.63	Green
Under iodine tank		
Spot 1 (*n*-hexane)	0.31	Yellow
Spot 2 (*n*-hexane)	0.37	Light brown
Spot 3 (*n*-hexane)	0.73	Brown
Spot 4 (methanol)	0.37	Dark brown
Spot 5 (methanol)	0.72	Brown
Spot 6 (aqueous)	0.42	Brown
Spot 7 (aqueous)	0.72	Brown

The calculated Rf values for spots observed under daylight and iodine tank for *n*-hexane, methanol and aqueous extracts as shown in Table 2 correspond to the standard Rf value for flavonoids [15]. Also, the Rf values calculated for spots observed under iodine tank for aqueous extract correspond to the standard Rf value for phenols [16], while the Rf values calculated for spots observed under UV for methanol extract, *n*-hexane extract under iodine tank and for methanol extract under daylight visualization, correspond to the standard RF value for steroids [17]. Furthermore, the Rf values calculated for spots observed under UV for aqueous extract, and for methanol extract correspond to the

standard Rf value for saponins [18]. Again, the Rf values calculated for spots observed under iodine tank for *n*-hexane extract, and for spots observed under UV for methanol extract and for spots observed under daylight visualization for aqueous extract correspond to the standard Rf value for terpenes [19]. The Rf value calculated for spot observed under UV for methanol extract corresponds to the standard Rf value for glycosides [20], while the Rf value calculated for spot observed under iodine tank for *n*-hexane extract corresponds to the standard Rf value for tannins [21].

Table 3. Mean zone of inhibition of aqueous extract of *P. nigrescens* on growth inhibition of test bacteria (mm).

Extract concentration (mg/mL)	*P. spp*	*S. aureus*	*K. pneumoniae*	*E. coli*
200	1.33±1.15^{ab}	5.00±0.00^{b}	8.00±0.00^{b}	1.33±1.15^{a}
400	2.60±0.57^{b}	6.33±0.57^{b}	10.00±1.00^{c}	5.33±0.57^{b}
600	6.33±0.57^{c}	10.00±0.00^{c}	11.33±0.57^{c}	5.60±0.57^{b}
Control (+)	18.60±2.30^{d}	27.00±1.00^{c}	17.33±1.52^{d}	34.60±3.51^{d}
Control (-)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

*Values are mean ± SD of three replicates. Values with different superscripts are significantly different at $p \leq 0.05$ (Duncan multiple range test).

Table 4. Mean zone of inhibition of *n*-hexane extract of *P. nigrescens* on growth inhibition of test bacteria (mm)

Extract concentration (mg/mL)	*P. spp*	*S. aureus*	*K. pneumoniae*	*E. coli*
200	7.33±1.15^{a}	6.33±0.57^{a}	10.60±0.57^{a}	5.60±1.15^{a}
400	8.66±0.57^{ab}	8.66±0.57^{ab}	12.33±0.57^{b}	6.60±0.57^{ab}
600	10.33±0.57^{b}	10.00±1.00^{b}	15.67±0.57^{c}	7.60±2.39^{b}
Control (+)	18.60±2.30^{c}	26.67±1.57^{c}	17.33±1.52^{d}	34.60±3.51^{c}
Control (-)	10.00±0.57^{b}	10.00±0.00^{b}	10.00±0.00^{a}	10.60±0.57^{b}

Table 5. Mean of zone of inhibition of methanol extract of *P. nigrescens* on growth inhibition of test bacteria (mm)

Extract concentration (mg/mL)	*P. spp*	*S. aureus*	*K. pneumoniae*	*E. coli*
200	10.33±1.52^{b}	7.60±0.57^{b}	10.00±1.00^{b}	6.67±1.15^{b}
400	12.33±1.52^{b}	9.33±1.15^{c}	11.60±1.15^{b}	8.00±1.00^{b}
600	15.33±2.30^{c}	15.00±0.00^{c}	16.60±3.21^{c}	11.33±1.15^{d}
Control (+)	33.33±1.57^{d}	27.00±1.73^{d}	23.67±1.52^{d}	37.00±1.00^{e}
Control (-)	11.33+1.45^{d}	13.66+1.52^{b}	13.67+1.76^{d}	15.00+1.00^{b}

Table 6. Inhibition of methanol extract of *P. nigrescens* on growth inhibition of fungi *Aspergilllius flavus* (mm)

Extract concentration (mg/mL)	Inhibition 48 h (cm)	Inhibition 72 h (cm)	Percentage inhibition 48 h (%)	Percentage inhibition 72 h (%)
200	3.65±0.00^{d}	4.62±0.03^{d}	2.67±0.00^{b}	4.42±1.02^{b}
400	3.37±0.03^{c}	4.10±0.00^{c}	9.98±0.96^{c}	15.28±0.27^{c}
600	2.97±0.03^{b}	3.85±0.03^{b}	20.65±0.91^{d}	20.94±0.96^{d}
Control (+)	24.80±1.00^{a}	25.80±1.00^{a}	68.00±4.00^{a}	70.00±0.00^{a}
Control (-)	3.75±0.00^{e}	4.84±0.01^{e}	0.00±0.00^{a}	0.00±0.00^{a}

Table 7. Inhibition of aqueous extract of *P. nigrescens* on growth inhibition of fungi *Aspergillus flavus* (mm).

Extract concentration (mg/mL)	Inhibition 48 h	Inhibition 72 h	Percentage inhibition 48 h (%)	Percentage inhibition 72 h (%)
200	3.55±0.03^{c}	4.25±0.03^{d}	14.80±0.98^{b}	10.00±0.74^{b}
400	3.55±0.07^{c}	4.15±0.00^{c}	15.38±1.81^{b}	12.63±0.00^{c}
600	3.45±0.00^{b}	4.05±0.00^{b}	18.34±0.65^{c}	14.74±0.00^{d}
Control (+)	25.40±0.00^{a}	25.60±1.00^{a}	65.80±3.90^{a}	69.70±4.00^{a}
Control (-)	4.22±0.03^{d}	4.75±0.00^{e}	0.00±0.00^{a}	0.00±0.00^{a}

Table 8. Mean zone of inhibition of *n*-hexane extract of *P. nigrescens* on growth inhibition of fungi *Aspergillus flavus* (mm)

Extract concentration (mg/mL)	Inhibition 48 h (cm)	Inhibition 72 h (cm)	Percentage inhibition 48 h (%)	Percentage inhibition 72 h (%)
200	3.20±0.10^{c}	3.85±0.07^{d}	27.43±5.61^{b}	13.45±1.50^{b}
400	2.90±0.00^{b}	3.62±0.11^{c}	30.48±2.94^{b}	18.50±2.36^{c}
600	2.65±0.07^{b}	3.30±0.00^{b}	36.42±4.40^{b}	25.80±0.00^{d}
Control (+)	25.40±1.00^{a}	25.60±1.00^{a}	68.00±3.80^{a}	69.00±4.00^{a}
Control (-)	4.20±0.20^{d}	4.45±0.00^{e}	0.00±0.00^{a}	0.00±0.00^{a}

The effect of growth inhibition of Parquetina nigrescenson test bacteria pathogens. Differences in inhibitory activities observed in the various concentrations of *n*-hexane, methanol and aqueous extracts of the studied plant can be linked to the extraction ability of the solvents employed [22]. Hence, the methanol was able to extract and dissolved the antimicrobial metabolites present in the leaves of *P. nigrescens*. The fully dissolved antimicrobial agents were reproducible in all the concentrations considered,

as evident by the zones of inhibition more than in the aqueous and *n*-hexane extracts. There was a noticeable increase on the length of zone of inhibition of the test bacteria pathogens as concentration of the extract of *Parquetina nigrescens* increased in Table 3, 4, 5, therefore the inhibitory effect of *Parquetina nisgrescens* on test bacteria pathogens was concentration dependent. In all the extracts at 600 mg/mL (*n*-hexane, methanol and aqueous), *Klebsiella pneumonia* was most inhibited producing the largest mean zone of inhibition compared to other test bacteria pathogens, while *E. coli* was the least inhibited. The average zones of inhibition formed by all the plant extracts were significantly different at $p > 0.05$. Distilled water was used as negative control for both the methanol and aqueous extract because water was used to reconstitute the extract and showed no zone of inhibition. In Table 4, *n*-hexane was used as negative control for *n*-hexane extract and showed mean zone of inhibition of 10.33 ± 0.58^{b},10.00 ± 0.00^{b}, 10.00 ± 0.00^{a}, and 10.67 ± 0.58^{b}, when tested against test bacteria pathogens *Proteus spp., Staphylococcus aureus, Klebsiella pneunomiae* and *Escherichia coli*, respectively. Methanol and distilled water were both used as control for methanol extract when tested against bacteria pathogens; *Proteus spp, Staphylococcus aureus*, *Klebsiella pneunomiae* and *Escherichia coli*, respectively. Treatment with commercially available anti bacteria agent, Streptomycin showed the highest zone of inhibition when compared to the extract tested against the test bacteria pathogens. It was observed in all the three extracts that *Escherichia coli*, was the most inhibited by the treatment (drug) and *Klebsiella pneunomiae* was the least inhibited. So, the strong activity of both the aqueous, methanol and *n*-hexane extracts indicates that the plant can be effective against skin and sexually transmitted infections.

The effect of growth inhibition of Parquetina nigrscens on fungi Aspergillus flavus and Candida albicans. There was a noticeable reduction on the length of growth of the fungi as the concentrations of the extracts of *Parquetina nigrscens* increased. The length and breadth of growth of the fungi were measured within the incubation period of 3-5 days. Inhibitory effect of *Parquetina nigrscens* on the fungi was determined at different concentrations: 200 mg/mL, 400 mg/mL, and 600 mg/mL. PDA with no botanical extract was used as negative control. The results of the growth of *Aspergillus flavus* when treated with different concentrations of the extract showed a remarkable decrease was observed in Table 6, 7 and 8. This proves the antifungal effect of *Parquetina nigrescens*. In Table 6, at 72 hours, the growth length of fungi when treated with 200 mg/mL, 400 mg/mL, 600 mg/mL of methanol, aqueous and *n*-hexane extracts were 4.4%, 15.3% and 21.0%; 10%, 12.6%, 14.7% and 13.5%, 18.5% and 25.8% percentage growth inhibition respectively, and with the control, fluconazole in place of the extracts showed percentage growth inhibition range of 68 - 70%. The extracts of the leaves of *P. nigrescens* did not show any inhibition on *Candida albicans* while the control showed inhibition within the range of 68 - 70%.

The results agree essentially with several investigations and reports that *Parquetina nigrescens* contains antimicrobial substances [23 - 26] but failed to agree with [27] that stated that *Parquetina nigrescens* could not inhibit *E. coli*. This could be due to the low concentration used in the study compared with high concentration in the current work, since *E. coli* has a slimy protective layer, it needs high concentration of the extract to be able to inhibit the bacteria. It may also be due to contamination of the extracts during preparation which could lead to resistance of *E. coli* to the extracts. Also, the differences in ecological, environmental and geographical factors may be unfavorable for the *E. coli* to thrive as well. These findings also did not agree with [28] that found out that the leaf extract could inhibit *Candida albicans*, and this could be due to differences in ecological, environmental and geographical factors unfavorable for the fungi *candida albicans* to thrive well. It also agrees with [29] that stated that methanol extracts of the plant could extract more bioactive compounds responsible for antimicrobial activity than aqueous extract. The methanol extracts of the plant in this study produced larger zones of inhibition than aqueous extract as well.

The random selection of the extracts doses were based on the fact finding to know if a lesser dose will work the same like a higher dose in the *n*-hexane, methanol and aqueous extracts. A particular dose of plant extracts is not considered for therapeutic applications to avoid side effect after a brilliant pharmacological activity of any plant extract, dose selection is necessary for safety application [28].

4. Conclusions

The high antimicrobial effect of the extracts may be due to the synergistic activity between the secondary metabolic compounds such as steroids, phenols, tannins, glycosides, terpenes, flavonoids found in the leaves of *Parquetina nigrescens* which could be useful chemotherapeutic agents against infections arising from the activities of these microorganisms. TLC profiling further confirmed the possible presence of steroids, glycosides, terpenes, saponins, flavonoids, tannins and phenols found in the extracts by comparing the RF of corresponding spot with that of standards. These findings also suggest that *Parquetina nigrescens* could be a potential source of natural antioxidant due to the presence of steroids, flavonoids and phenols having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases which could lead to dysfunctionality of sexual organ as well as supporting the acclaimed aphrodisiac use of the plant in folk medicine.

Conflict of interest statement

The authors wish to declare that there is no conflict of interest.

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