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Assessment of pharmacological properties of root extracts from *Ficus capensis*

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Abstract: Evaluation of pharmacological properties of root extracts from fig tree was conducted using different organic solvents in the extraction process. Six (6) samples were prepared for experimental assessment of their cytotoxicity, brine shrimp lethality, antioxidant, total-antioxidant and antibacterial activities using conventional methods with slight modifications. Results obtained from the analyses revealed their potency against bacterial strains, brine shrimp larvae and radical/electron scavenging activities. All the tested extracts exhibited potent cytotoxicity with sample A at LC_{50} values of 0.20µg/mL showed higher toxicity than control (potassium dichromate LC_{50} = 7.23µg/mL). Among the tested samples, sample F revealed the highest inhibitory property with IC_{50} value of 23.10µg/mL. Samples A and D exhibited significant inhibitory activity with IC_{50} values of 116.50µg/mL and 355.30µg/mL respectively. Isolated compounds from methanol extracts (sample E and F) showed better performances as antioxidant-agent against pathogens associated to attack by free radical mechanism. With current trend of drug resistance by many microorganisms, new compounds have been isolated from the root of *F. capensis* that showed better performance against cultured: *Escherichia coli*, *Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumonia* at different concentrations.

Keywords: antibacterial, cytotoxicity, *Ficus capensis*, lethality, root extracts

Introduction

Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Eighty percent (80%) of present day medicines are directly or indirectly derived from plants known to have been investigated pharmacologically out of thousand species of higher plants growing on earth. *Ficus* is of the Mulberry family (Moraceae) and possibly the most illustrious member of this family's genera. The fruits and trees of the *Ficus* genus are among the oldest and most successful species of higher plants on earth (Rahman and Khanon, 2013). They are seen throughout the tropics and

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few in the semi warm temperate zone. They are mostly evergreen while some are endemic to areas outside of the tropics. *Ficus capensis* with synonyms as *Ficus sur,* cape fig and *Sycomorus capensis* is a fig tree with medicinal properties found in the terrestrial zones mostly along the river banks. *F. capensis* is locally referred to as "Yandi" (Hausa), "Opoto" (Yoruba), "Rima bichehi" (Fulani), "Obada" (Edo) and "Akokoro" (Igbo) belongs to the family Moraceae, and has been regarded as an underutilized plant (Njoku-Oji *et al*., 2015). It is an evergreen tree with spreading branches and roots and about medium sized tree of 6-9 meters high (Solomon *et al.,* 2011). The leaves are broad and green and the tree produces fruit all through the year in a single or branched raceme along the branches (Igwe *et al.,* 2016). The leaves are used as vegetable with a reported blood boosting effect and an anti-sickling effect of red blood cells (Igwe *et al.,* 2016). The reported medicinal properties of its extract include being used in the treatment of diarrhea and dysentery, sexually transmitted diseases, chest ailments, leprosy, convulsion, pain, tuberculosis, anaemia and wound (Adebayo-Tayo and Odeniyi, 2017). It is also used in circumcision, wound dressing, leprosy and epilepsy treatment (Igwe *et al*., 2016). Antioxidants are compounds which inhibit the oxidation processes that occur under the influence of atmospheric oxygen or reactive oxygen species. They are involved in the defense mechanism of the organism against the pathologies associated to the attack by free radicals. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs (Ayad *et al.,* 2017). Pathogens frequently display resistance to current drugs, which frequently lack selectivity/efficacy and have detrimental side effects (Kasumbwe *et al*., 2014). With the continuous use of antibiotics, microorganisms have become resistant, in addition to these problems; antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut, mucosal microorganism, immuno-suppression and allergic reactions has created immense clinical problem in the treatment of infectious diseases. Therefore, there is a need to search for new potential effective bioactive compounds against pathogenic bacteria and fungi (Ang *et al.,* 2019) The aim of this research is to study pharmacological properties of *Ficus capensis* root by analyzing its cytotoxicity, antioxidant and antibacterial potentials.

Methodology

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Root of *Ficus capensis* were collected at Kazaure Local Government, Jigawa State. Fresh leaves of the plant were identified by the herbarium curator from Botany Department, Bayero University, Kano. Following the methods described by Banu and Catherine (2015), the fresh roots of *F. capensis* were shade dried in the laboratory. The dried sample was thereafter coarsely ground, sieved and weighed. The dried powder (700.0g) was defatted using n-hexane (2.5 L) for 14 days. The residues were percolated with ethanol (2.5L) for 14days. The extract was filtered, concentrated using rotary evaporator to yield ethanol extract (Sample A).

Fractionation of Ethanol Extract

Ethanol extract (16.0g) was macerated using *n*-hexane, chloroform and 60% aqueous methanol. The fractions were concentrated using rotary evaporator which afforded n-hexane fraction (Sample B) and chloroform fraction (Sample C) while the 60% aqueous methanol was further subjected to freeze-dryer to obtain aqueous methanol fraction (Sample D). The weight and appearance of the respective fractions were recorded in accordance with the method described by Kumar *et al*., (2013).

Thin Layer Chromatography (TLC) of the Samples

TLC was performed by spotting the sample using capillary tube on precoated aluminum silica gel plate (4.0 ×5.0) cm^2. Each plate was developed in acetone solvent system and allowed to dry. The plates were visualized under UV shorter and longer wavelength (254nm and 365nm) and stained with iodine through iodine crystal exposure (Kumar *et al*., 2013).

Column Chromatographic Separation of Aqueous Methanol Fraction

A silica gel column (150.0g silica, column size 2.30cm x 121.0cm) was packed and washed with n-hexane. The sample, aqueous methanol (3.82g) was mixed with silica gel until non-sticky homogenous powder form. The sample was then loaded on to the column. A protecting layer is formed with fresh silica on top of the sample. Elution of the sample was carried using *n*-hexane, *n*-hexane/ethyl acetate (Sample E) (90:10, 80:20, 75:25, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 30:70, 20:80, 10:90), 100% ethyl acetate, ethyl acetate/methanol (90:10, 80:20, 70:30, 50:50) and methanol to afford 225

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fractions (Sample F). Similar fractions were pooled on the basis of TLC profiles (Bajpai *et al.,* 2016 and Dar *et al.,* 2017).

Cytotoxicity Assay against Brine Shrimp

Brine shrimp eggs (Artemix, GmbH & Co., Germany), *Artemia salina* was hatched in distilled water (16.0g in 500mL). After 24 hours incubation at room temperature, the larvae were attracted to one side of the vessel with a light source and collected using pipette. Larvae were separated from eggs by aliquoting them 3 times in small beakers containing seawater (Atta-ur-Rahman *et al.,* 2005).

Brine Shrimp Assay

Toxicity of the extract was monitored by the brine shrimp lethality test according to the method reported by Sarah *et al.,* (2017) and Atta-ur-Rahman *et al.,* (2005) with slight modification. Each of the extracts (1.0mg/mL) was dissolved in methanol, from which 5000, 500 and 50µL of each solution was transferred into vials corresponding to 1000, 100 and 10µg/mL respectively. A negative control was prepared as a drug-free and potassium dichromate was used as positive control. Survivors were counted after 24 hours and LC_{50} was determined by Probit analysis of SPSS version 20.

Antioxidant Activity; (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The free radical scavenging activity of the root extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method described by Sundari *et al* (2017) with slight modification. Each sample of stock solutions (1.0mg/mL) was diluted to final concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.63 and 7.82µg/mL. The absorbance of the mixtures was measured at 517nm while ascorbic acid was used as a standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity and vice versa. Inhibitions of DPPH radical in percent (I%) was calculated using the formula:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100.$$

Where; A_{blank} = the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} = the absorbance values of the test compounds.

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The sample concentration that provides 50% inhibition (IC_{50}) was determined using SPSS 20.

Total Antioxidant Activity by Phosphomolybdenum Assay

Total antioxidant capacity of sample was determined according to phosphomolybdate method by Ayad *et al.*, (2017). 180µL of reagent solution (6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate) was added to 20µL of each extract (0.1mg/mL). The absorbance was measured against blank at 695nm using a UV/visible spectrophotometer, after incubation for 90 minute at 95°C. Antioxidant capacity of the extracts was expressed as µg ascorbic acid equivalent per mg (µg AAE/mg).

Antibacterial Activity

Nutrient broth (20.0g/L), McFarland barium sulphate turbidity standard of 0.5, bacterial culture; *Escherichia coli, Proteus mirabilis, Pseudomonas* and *Klebsiella pneumonia* were used in the analysis. Bacterial activity was determined following the standard broth micro-dilution method as described by Stanley (2012). A stock solution of the standard antibiotic (gentamicin) and samples were 2-fold serially diluted until a concentration in the range of 1000-125µg/mL was obtained. The bacterial cultures were adjusted to 10^5 colony-forming unit (CFU/mL) inoculum size, employing the standard 0.5 MacFarland scale. 0.1mL of culture was added to each test tube which contains culture medium and the dilute samples. These were carried out in triplicate. The test tubes were incubated at 37°C for 24 h. The MIC value was determined as the lowest concentration of the sample assayed that does not show turbidity. Minimum Bactericidal Concentration (MBC) was determined by sub-culturing all the concentrations showing no growth on nutrient agar using streak method. Growth of each organism on each concentration was checked after 24 hours' incubation at 37ºC.

Findings

Ficus capensis root (700.0g) was extracted with 98% ethanol to yield 22.5g of darkish green extract with percentage recovery of 3.22%. The ethanol extract (16.0g) was fractionated with *n*-hexane, chloroform, 60% aqueous methanol to yield their respective samples A, B, C, and D (Figure 1). The extracts were subjected to brine shrimp lethality test (BSLT) to assess their level of toxicity. The toxicity was expressed as % Mortality

and 50% Lethal Concentration (LC_{50}) in µg/mL. All the tested extracts (Table 1) exhibited potent cytotoxicity with the ethanol extract, sample A at LC_{50} values of 0.20µg/mL showed higher toxicity than control; potassium dichromate (LC_{50} = 7.23µg/mL). DPPH radical scavenging activity of the extracts and isolated compounds were expressed based on percentage inhibition and 50% inhibitory concentration in µg/mL (Table 2). Among the tested samples, sample F has the highest inhibitory property with IC_{50} value of 23.10µg/mL. Samples A and D exhibited a significant inhibitory activity with IC_{50} values of 116.50µg/mL and 355.30µg/mL respectively. The total antioxidant activity of the samples was evaluated using phosphomolybdate technique. A reduction of molybdenum (VI) to molybdenum (V) by antioxidant was monitored by the formation of green color of Mo(V). From the calibration curve of ascorbic acid (Figure 2), the tested samples indicated relatively low antioxidant capacity in the range of 16.50 to 23.33µgAAE/mg (Table 3). The activity of bacterial strains; *E. coli, P. aeruginosa, P.. mirabilis and K.. pneumonia* were evaluated for the possible inhibitory properties of extracts and isolated compounds from the root of *F. capensis*. Micro-dilution technique was employed in which the activity was expressed as Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in µg/mL (Table 4). The antibacterial effect of the test samples were investigated at concentration levels of 1000, 500, 250, 125µg/mL. All the samples were found to have MIC values of 250µg/mL. Further evaluation of their respective MBC revealed different grades of antibacterial effect. Sample A and B did well against *P. mirabilis,* sample D had better performance on *P. aerugonosa* while sample F did better against *E. coli* but antibacterial activities of other test samples recorded a bacteriostatic effect.

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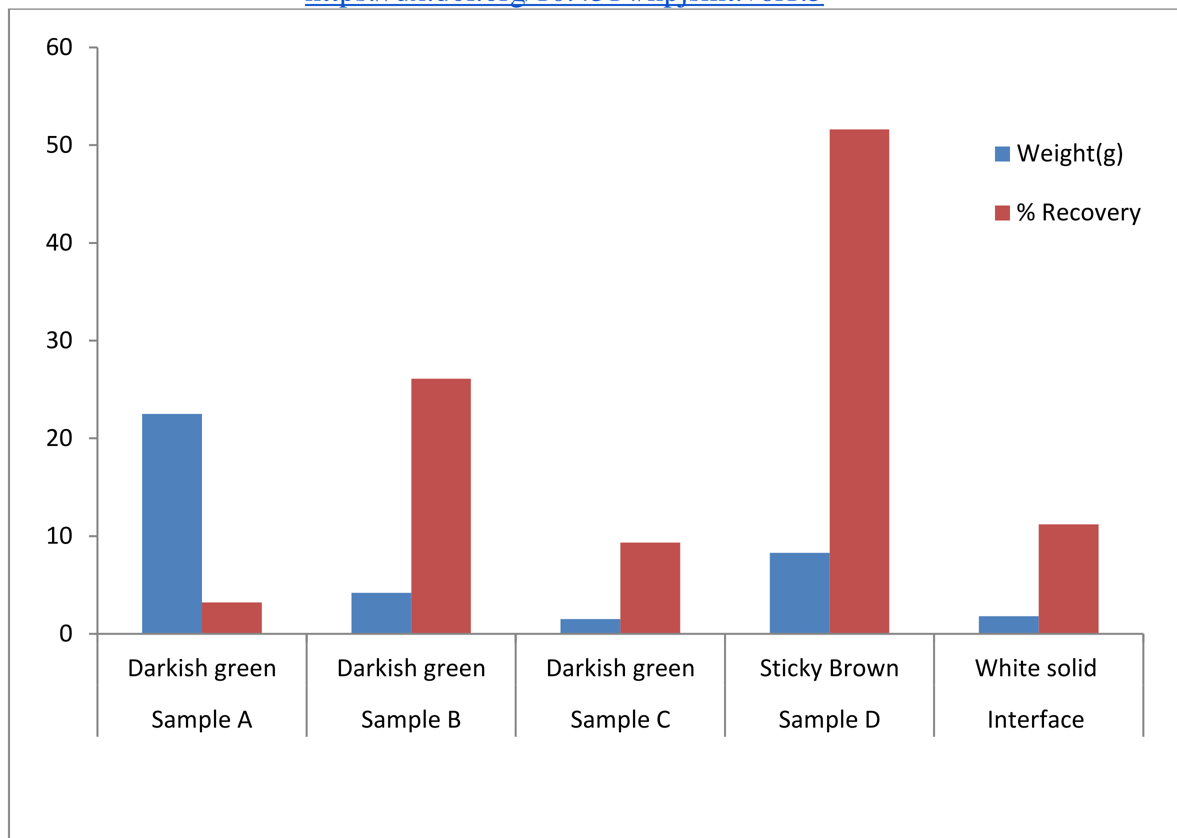

Figure 1: Mass and percentage yield of *F. capensis* root extracts

Table 1: Brine shrimp lethality result of *Ficus capensis* root extracts

Sample	Conc. (µg/mL)	Replica	Total nauplii	Dead nauplii	%Mortality	LC_{50}(µg/mL)
A	1000	3	10	10	100	0.20
	100	3	10	9	90	
	10	3	10	9	90	
B	1000	3	10	9	90	121.78
	100	3	10	8	80	
	10	3	10	6	60	
C	1000	3	10	10	100	126.47
	100	3	10	3	30	
	10	3	10	1	10	
D	1000	3	10	10	100	104.58
	100	3	10	5	50	

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	10	3	10	0	0	
Potassium	1000	3	10	10	100	7.23
dichromate	100	3	10	10	100	
	10	3	10	6	60	

Table 2: IC_{50} result of *Ficus capensis* root extract and isolated fractions

Conc. (µg/mL)	1000	500	250	125	62.5	31.25	15.6	7.8	IC_{50} (µg/mL)
Sample A	82.5	55.9	48.8	43.3	34.7	33.1	19.8	17.4	116.50
Sample B	43.5	36.2	33.0	35.2	24.2	26.	26.2	13.5	>1000
Sample C	34.2	32.1	36.6	32.5	30.2	34.3	25.7	31.5	>1000
Sample D	75.9	51.8	35.9	34.6	26.1	24.6	27.9	27.0	355.30
Sample E	45.1	37.4	44.0	42.8	44.3	34.7	41.8	41.0	>1000
Sample F	96.1	87.4	65.5	60.5	51.2	58.5	51.5	41.0	23.10
Ascorbic acid	97.5	97.0	97.5	97.3	97.6	95.3	94.1	64.5	0.75

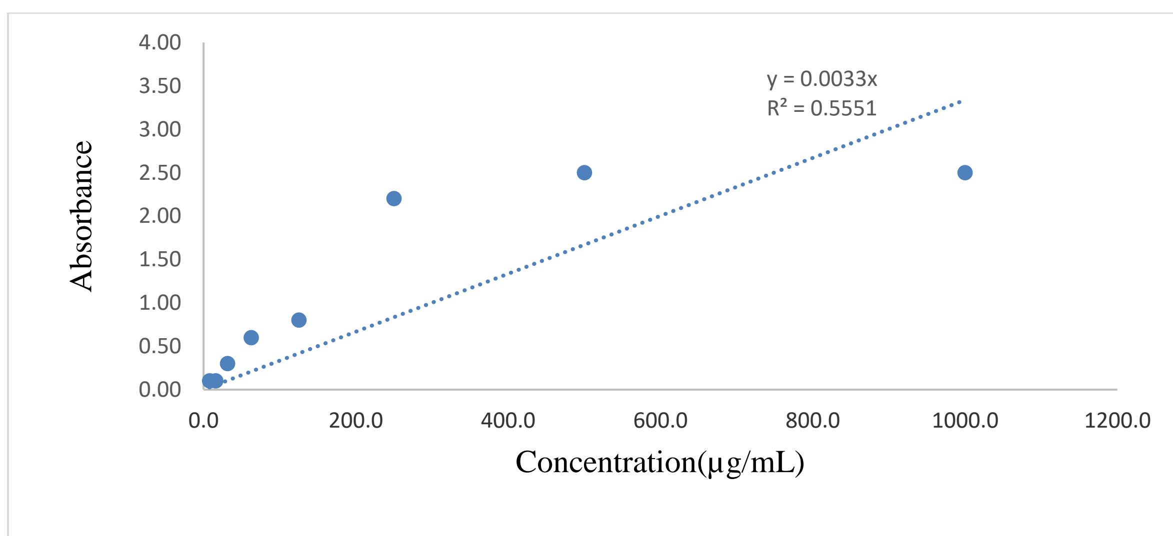

Figure 2: Calibration curve of ascorbic acid standard

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Table 3: Total antioxidant activity of *F. capensis* root extracts and isolated fractions

Samples	TAA (µgAAE/mg)
A	22.10 ± 0.0036
B	23.33 ± 0.0013
C	21.40 ± 0.0040
D	16.50 ± 0.0033
E	19.94 ± 0.0027
F	22.00 ± 0.0024

Values are Mean±SD (n=3)

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) results of *F. capensis* root extracts and isolated fractions

Samples	MIC/MBC (µg/ml)	Microorganisms			
		E_8	K_4	P_r	P_s
A	MIC	250	250	250	250
	MBC	1000	1000	250	1000
B	MIC	250	250	250	250
	MBC	1000	1000	250	1000
C	MIC	500	250	250	250
	MBC	500	1000	1000	1000
D	MIC	250	250	250	250
	MBC	1000	1000	1000	250
E	MIC	250	250	250	250
	MBC	500	1000	1000	1000

F	MIC	250	250	250	250
	MBC	250	1000	1000	250
Gentamicin	MIC	125	125	125	125
	MBC	125	125	125	125

E_8: *Escherichia coli*, Ps: *Pseudomonas aeruginosa,* Pr*: Proteus mirabilis* and K_4: *Klebsiella pneumonia.*

The percentage recovery of crude ethanol extract (3.22%) was based on the mass of dried and ground plant material while percentage recovery of fractionated extracts was obtained by weighing crude ethanol extract (Figure 1). The highest recovery was obtained in aqueous methanol extract (51.61%) followed by n-hexane (26.12%) whereas the chloroform extract (9.33%) had the lowest recovery percentage. Using different solvents for extraction in study is to establish which solvent enhances high solubility which in turn produced better separation of compounds in each extract.

Hamidi *et al*. (2014) reported the Meyer's toxicity index of extracts with LC_{50} < 1000µg/mL are considered as toxic but extract with LC_{50} > 1000µg/mL are considered as non-toxic while Clarkson's toxicity assessment of plant extract is as thus; LC_{50} above 1000µg/mL is regarded as non-toxic, LC_{50} of 500 - 1000µg/mL is low toxic, extracts with LC_{50} of 100 - 500µg/mL is medium toxic whereas extracts with LC_{50} of 0 - 100µg/mL is highly toxic. Going by these two classifications (Meyer's and Clarkson's), the extracts from this study are toxic, Sample A showed most potent toxic effect at LC_{50} = 0.20µg/mL followed by sample D (LC_{50} = 104.58µg/mL) which was closely followed by sample B (LC_{50} = 121.78µg/mL) whereas sample C (LC_{50} = 126.48µg/mL) had the least toxic among samples analyzed as indicated in Table 1. The results revealed the extracts prepared are potent enough to destroy the pathogens within their hosts.

Antioxidant compounds quench DPPH free radical by providing hydrogen atoms or by electron-donation resulting in absorbance at 517nm and the more rapidly the absorbance decreases, the more potent the antioxidant activity of the tested sample. The DPPH radical scavenging activity of samples A and D with IC_{50} value of 116.50µg/mL and 355.30µg/mL respectively were significant when compared with samples C and B with

IC_{50} values > 1000µg/mL. The decreases in activity might be attributed to decrease in polarity of solvents, low phenolic or flavonoid content or steric inaccessibility of large molecules as suggested by Ahmad *et al*., (2015). Sample F had the highest inhibitory property showed positive correlation with glycosidic nature of the compound as reported by Ang *et al*. (2019). On the other hand, sample E showed IC_{50} values > 1000µg/mL which might be attributed to fact that ortho-substituted compound is almost inactive in solvent while phenolic is moderately active as suggested by Ang *et al*. (2019). The higher the value of total antioxidant activity, the more effective is the antioxidant activity of a tested sample. Phosphomolybedate assay involves electron transfer mechanism where many natural products including flavonoids and phenols can cause the reduction of Mo(VI) to Mo(V) as reported by Akachukwu and Uchegbu (2016) and Ahmad *et al*., (2015). The total antioxidant of the samples prepared at 0.1mg/mL was in the range of 5.14 - 6.30mgAAE/g. This low antioxidant activity values might be due to low phenolic or flavonoid content coupled with steric inaccessibility of large molecules as suggested by Ahmad *et al*., (2015). All the samples were found to have MIC values of 250µg/mL. Further evaluation of their respective MBC indicated bactericidal effects; sample A and B revealed higher effects against *P. mirabilis,* sample D performed well against *P. aerugonosa* whereas sample F performed well against *E. coli*. This suggests effectiveness of the extracts as therapeutic agents against many pathogens. This result is collaborated to the findings reported by Adebayo-Tayo and Odeniyi (2017), Igwe *et al*. (2016) and Solomon *et al*. (2011) on other parts of the plant (*Ficus capensis*) with the exception of its root.

Conclusion

Experimental evaluation of cytotoxicity, brine shrimp lethality, antioxidant, total-antioxidant and antibacterial activities of *Ficus capensis* root extracts and its fractions were assayed using conventional methods with slight modifications. Results obtained from the analyses revealed their potency against bacterial strains, brine shrimp larvae and radical/electron scavenging activities. With the results obtained in this study, *F. capensis* root extracts showed excellent potential in ameliorating ailments related to pathogenic bacteria. Isolated pure compounds from methanol extract (sample E and F) showed better performances as an antioxidant agent compared to samples (A, B and C) which helps in defense mechanism against the pathologies associated to the attack by

free radicals. All the tested samples exhibited potent cytotoxicity with sample A performing better than control sample (potassium dichromate). Other samples showed good results but less than control. With current trend of drug resistances by many microorganisms, new compounds have been isolated from the root of *F. capensis* that showed better performance against cultured: *Escherichia coli*, *Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumonia* at different concentrations. Isolated fractions of samples E and F showed new potential bioactive compounds that can curtail the problems of pathogenic bacteria which can be used to correct the incidence of drug resistances in the area of drug development.

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