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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *URENA LOBATA* AND *EMILIA COCCINEA* METHANOLIC STEMS EXTRACTS.

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ABSTRACT.

This study aimed to investigate possible antioxidant and antibacterial activities of methanolic extracts of *Urena lobata* (Uls) and *Emilia coccinea* (Ecs) stem. Phytochemical analysis showed alkaloids, phytosterols, tannins, saponins, phenolic compounds and flavonoids.

Antimicrobial activity was evaluated and *Urena lobata* stem extract has antibacterial activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains while *Emilia coccinea* stem shows positive effect only on Staphylococcus *aureus* strain. MIC was 6.25 and 12.5 mg/ml respectively for Ecs and Uls and MBC was 25 mg/ml for both extracts.

Total phenolics contents with total flavonol and flavonoid; ferric reducing antioxidant power, phosphomolybdum assay, DPPH (1,1-diphenyl-2-picrylhydrazyl), hydroxyl and ABTS radical-scavenging activities were also determined. IC_{50} analysis showed that Uls is the best ABTS and hydroxyl scavenging respectively with 20.12 mg/ml and 4.488 mg/ml upper than vitamin C (11.194 and 3.424 mg/ml). However Ecs showed the best DPPH radical scavenging

(5.272 mg/ml) also upper than the one issue from Vit. C (4.697 mg/ml). ECs showed the highest total phenolics contents (151.33 ± 26.86 CAE/gram of dry extract), flavonoid (6.0 ± 0.8 QE/gram of dry extract) while ULs showed rather the highest flavonol content (49.33 ±5.77 CAE/ gram of dry extract). According to ferric reducing antioxidant power and phosphomolybdum assay, Ecs and Uls showed respectively the best activity with 0.20 ± 0.007 % and 0.22 ± 0.01 %, suggesting that these extract might contribute antioxidant activities of these plants.

These findings mean that *Urena lobata* (Uls) showed antiradical and antibacterial activities.

Keys words: antioxidant, antibacterial, extracts, *Urena lobata*, *Emilia coccinea*, stems.

INTRODUCTION

Plants such as herbs have long been used in traditional medicine in various cultures throughout the world. Over 100 plant species are consumed worldwide as vegetables, but only about 20 of them are grown globally and account for most of the vegetables produced and consumed [1]. Various types of plants have been used not only for dietary supplements but also as traditional folk treatments for many health problems [2].The use of folk medicine is widespread and herbaceous plants comprise an important source of bioactive compounds possessing potent biological properties. They have played a significant role in traditional medicine since ancient times and still represent an important source of natural antioxidants that might lead to the development of novel drugs [3]. Antioxidant activity is a fundamental property important for human life. Many biological functions, including anti-mutagenicity, anti-carcinogenicity, and anti-aging, may originate from this property [4]. The increased consumption of herbaceous plants has been widely promoted because of the health benefits of many non-nutrient phytochemicals associated with health maintenance and prevention of chronic diseases and cancers. As our understanding of the role of free radicals in human diseases has deepened, antioxidants have attracted broader interest because of their role in inhibiting free radical reactions and their help in protecting the human body against damage by reactive oxygen species [5]. However, herbaceous plants differ in the types and levels of antioxidants they contain. The synergies and antagonisms of antioxidants in crude mixtures add complexity in attempts to explain their antioxidant capacity. Some phenolics are ubiquitous compounds found in all plants as secondary metabolites [6]. Numerous groups of phytochemicals in plants are recognized for their antioxidant activity [7].Crude extracts of

fruits, herbs, vegetables, cereals, and other plant materials are rich in phenols and are increasingly of interest to the food industry because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food [8]. Phenolic compounds found have antioxidant effects, therefore, their ingestion may help to prevent in vivo oxidative damage, like that which occurs in lipid peroxidation in association with cancer and premature ageing [9].

Some medical plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine and natural therapies for many thousands of years. It is generally considered that compounds produced naturally, rather than synthetically, will be biodegraded more easily and therefore be more environmentally acceptable. Thus, natural antioxidants, antibacterial, cytotoxic, antiviral, fungicidal agents and nutrients have gained popularity in recent years, and their use and positive image among consumers are spreading. In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [10]. In order to find new therapeutic agents, plants such as *Urena lobata* and *Emilia coccinea* that have antimicrobial or antioxidant activity have attracted our attention.

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MATERIAL AND METHODS

Plant material: The stem of *Urena lobata* and *Emilia coccinea* were collected at the Kala Mountain in the Center region of Cameroon. They were respectively authenticated by M. NANA, a botanist at the National Herbarium of Cameroon, in comparison to the voucher specimens as numbers 6976/SFR/CAM and 19901/HNC.

Preparation of plant extracts: The collected part were dried at ambient temperature, crushed and ground into powder using an electrical grinder and stored in desiccators. The yield of extracts was 5.10% and 7.60% respectively for the *Urena lobata* and *Emilia coccinea* extracts. The powders were then macerated for 48 h in pure methanol and the mixtures were filtered using a Buchner funnel and Whatman No 1 filter paper. This process was repeated once on the residue. The filtrate was concentrated using a rotavapor and the solution was dried in the oven at 55°C for two days. Each crude extract obtained was labeled using the following codes: Uls for *Urena lobata* and Ecs, for *Emilia coccinea*. The different samples were then kept at 4°C. Prior to the experimentation, the solutions of the both plant extracts

were dissolved using methanol 2 % to different dilutions (25, 50, 75, 150, 300 µg/mL) of each.

Phytochemical screening: The quanlitative methods described by [11, 12] were used to evaluate the presence of some phytochemical compounds such as phenols, polyphenols, flavonoids, tannin, saponin, flavonoids, steroid and terpen.

In vitro antibacterial activity

Preparation of the bacterial inoculum: For each tested micro-organism, overnight cultures of bacterial colonies seeded on Mueller Hinton Agar (Fortress Diagnostics Limited U.K) and incubated at 37°C were suspended in 5 ml saline water in test tubes. This suspension was read thereafter with a spectrophotometer at 625 nm. When the optical density was between 0.08 and 0.13, the bacterial load was 108 CFU/mL (0.5 McFarland). After a 100th dilution, the bacterial load was 106 CFU/ml [13].

Preliminary sensitivity test of the strains to the extracts: The preliminary tests of sensitivity of the bacterial strains to the various extracts were carried out as recommended by [14]. 100 µl of each bacterial inoculum was inoculated on Mueller Hinton agar (Fortress Diagnostics Limited U.K). The Petri dishes were then allowed to dry at ambient temperature under a fumes cupboard for 15 min. 6 mm wells were bored in the agar and the bottom of each well plugged with a drop of Mueller Hinton agar to limit the diffusion of the extracts from below. Fixed volumes of 50 µl of the stock solutions of each extracts and gentamicin respectively concentrated at 50 mg/ml and 1 mg/ml were then introduced into each well. After a pre diffusion time of 15 min of the antibacterial substances to be tested at ambient temperature, the Petri dishes were incubated at 37°C for 24 h. The inhibition diameters round of each well was measured using a sliding caliper. Each test was carried out in triplicate and the inhibition diameters expressed mean ± standard deviation.

Determination of the inhibition parameters: MIC and MBC: The inhibition parameters of bacterial growth were evaluated according to the M27-A9 guideline described by [15]. This involved preparing double dilutions of tested substances in 100 µl of glucose supplemented nutrient broth (GNB) medium (Acumedia Manufacturers) into the wells of a microtiter. The range of final concentrations tested were 0.25 to 0.097 mg/ml for each plant extract and 0.250 to 0.00097 mg/ml for gentamicin (Brunhild Pharmaceutical Private Limited). Each serial dilution was performed in triplicate. The bacterial inoculum was prepared at 106 CFU/mL using McFarland. Volumes of 100 µl of this inoculum were distributed to all the wells of the

microtiter. A line of the plate without plant extract served as a control for the growth of the organism (negative control) and another (without plant extract and without inoculum) served as sterility testing medium (positive control). The microtitre plates were thereafter sealed with aluminum foil and incubated at 37°C for 24 h. After incubation, 40 µl of 2,3,5-triphenyl tetrazolium chloride (Sigma-Aldrich) concentrate at 0,2 mg/mL were introduced into each well. The MIC was defined as the smallest concentration of the extract for which there was no change in the initial yellowish color of the medium to red. The MBC were determined by subculture. 50 µl of the contents of wells greater than or equal to the MIC was introduced into 150 µl of fresh GNB. The microtitre plates were incubated for 48h at 37°C, thereafter revealed as earlier done. The smallest concentration for which no color change was observed and regarded as the minimum bactericidal concentration.

In vitro antioxidant activity

Folin-antioxidant capacity: Folin reagent diluted 10 times prior to utilization was used to measure the antioxidant capacity via polyphenol content [16]. 1mL of reagent was added to 10 µL of plant extracts. The absorbance at 750 nm was measured after 30 min using a spectrophotometer with catechin as the standard. The result was expressed as milligram of catechin equivalent per gram of sample (CAE/ g of extract).

DPPH free-radical scavenging assay: The antioxidant activity of extracts was measured in terms of radical scavenging ability, according to the DPPH method [17]. In the procedure, DPPH free radical (violet color) was reduced by antioxidant. The stronger antioxidant present in the plant extract, the fainter the solution color was. Scavenging activity against the DPPH free radical was studied as follows: 20 µL of extract was introduced into 2 mL of a methanolic solution of DPPH (0.3 mM) and kept in the dark for 30 min. The extract was replaced by methanol for the control and catechin was used as the standard. The absorbance was then spectrophotometrically read at 517 nm. The antioxidant content and inhibition rates of DPPH radical were calculated as milligram of catechin equivalent per gram of sample (CAE/ g of extract).

FRAP assay: The antioxidant capacity of each sample was estimated according to the procedure described by [18]. Briefly, 75µL of extracts solution was added to 2 mL of FRAP reagent. The free radical scavenging activity was expressed as milligram of catechin equivalent per gram of sample (CAE/ g of extract).

ABTS free-radical scavenging assay: The ABTS solution was prepared by mixing 8 mM of ABTS with 3 mM of potassium persulfate in 25 mL of distilled water. The solution was maintained at room temperature in the darkness for 16 hours before use [19]. The $ABTS^+$ solution was diluted 10 times with 95% ethanol. Plant extracts (20 µL), was mixed with 1 mL of diluted $ABTS^+$ solution and incubated for 30 min at room temperature. The absorbance was read at 734 nm after 30 min against ethanol (95%) used as a blank.

The percentage of the radical scavenging activity of each extract was calculated from the equation below: Scavenging effect (%): $100\times (A_0-A_S)/A_0$; where A_o is the absorbance of the blank and A_s, the absorbance of the sample.

For each individual antioxidant assay, a catechin (1mM) aliquot was used to develop a standard curve and results were expressed as milligrams of catechin equivalent per gram of dried extract.

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity of the plant samples was determined as described by [20]. The reaction mixture consisted of FeCl3 (300 µM) and aliquots of extracts (2.5mg/mL -10mg/mL) in a final volume of 1 mL. All the reagents were dissolved in potassium phosphate buffer (20 mM, pH 7.4). After incubation at 37°C for 1 hour, 1 mL of TCA (2.8%) and TBA (1%) were added to the reaction mixture and incubated at 100 °C for 20 minutes. A control tube was prepared similarly except that the extract was replaced by methanol. The absorbance was read spectrophotometrically at 532 nm. The percentage of hydroxyl radical scavenging effects of plants extracts were calculated as follows: hydroxyl radical scavenging effect (%) = $[(Abs_1-Abs_2)/Abs1] \times 100$, where Abs_1 is the absorbance of the control and Abs_2, the absorbance of plant extract.

Determination of total flavonoid content: Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to a known method described by [21] using quercetin as a standard. A volume of 0.1 mL of plants extracts was added to 0.3 mL distilled water followed by 0.03 mL of $NaNO_2$ (5%). After 5 min at 25°C, 0.03 mL of $AlCl_3$ (10%) was added. After a further 5 min, the reaction mixture was mixed with 0.2 mL of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 mL with water and the absorbance was measured at 510 nm. The results were expressed as quercetin equivalent per gram of dried extract (QE/g dried extract).

Determination of total flavonols: Total flavonols in the plant extracts were estimated using the method described by [22] with slight modifications. In fact, to 2.0 mL of sample, 2.0 mL

of 2% of ethanolic solution of $AlCl_3$ and 3.0 mL (50 g/L) sodium acetate solutions were added. After 2.5 h of incubation at 20°C, the absorbance was read at 440 nm. The results were expressed as quercetin equivalent per gram of dried extract (QE/g dried extract).

Statistical analysis: The results were presented as mean ± SD of triplicate assays. Analyses of data were conducted using one-way Analysis of variance (ANOVA) followed by Kruskal wallis test and Dunnett's multiple test (SPSS program version 18.0 for Windows, IBM Corporation, New York, NY, USA). The Log probit was used to determinate the IC_{50} using the software XLstat version 7 (Addinsoft, New York, NY, USA) and to achieve the Pearson Correlation Analysis (PCA).The differences were considered as significant at $P<0.05$.

RESULTS

The result of phytochemical screening showed that both extracts contained compounds known to have antioxidant activity like phenols, polyphenols, flavonoids, saponin, steroid and terpen. *Urena lobata* extract as shown in table 1 did not contain tannin oppositely to Uls which contains those components.

From table 2 above, showing susceptibility test on some bacterial strains, we can observe that *Urena lobata* stem extract has antibacterial activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains while *Emilia coccinea* stem shows positive effect only on *Staphylococcus aureus* strain. It is important to notify that those anti bacterial activities were less than those obtained with gentamicin used here as reference molecule.

The results obtained for the inhibition parameters (Table 3) show that the MIC of the plant material was 6.25 and 12.5 mg/ml respectively for Ecs and Uls. The MBC was 25 mg/ml for both extracts.

The scavenging ability of DPPH free radical is widely used to analyze the antioxidant potential of naturally derived foods and plants. The DPPH radical scavenging potential of the different extracts is presented in Table 4. From this Table, all the extracts showed an inhibitory potential against DPPH free radical. The inhibitory percentages vary according for *Emilia coccinea* stem (Ec_s) from 21.98±5.81% to 55.74±8.67% at 300µg/ml and 35.45±2.91% to 52.31±2.01% for *Urena lobata (*Ul_s). In fact, these both extracts showed less DPPH activities compared to vitamin C at the same concentration. Both extracts showed the

significant lowest ($p < 0.05$) scavenging potential at the different concentrations compared to the vitamin C.

Several complementary methods have been proposed to assess the antioxidant activity of plant extracts and pure compounds. In vitro assays for the free radical scavenging capacity are usually based on the inactivation of radicals such as hydroxyl (OH) radicals. Table 5 presents the results of the OH radical scavenging activity of the extracts. At the lowest concentration of extracts (25 µg/mL), the scavenging properties of Ecs (63.89 ± 4.81%) is significant higher ($p < 0.05$) than those of Uls (16.67 ± 8.33%) and vitamin C (44.44 ± 9.62 %). As shown in table 5, Ec_s has the best OH radical scavenging activity compared to Ul_s.

Table 6 depicts the results of ABTS antioxidant activities of the different stem extracts. As the concentration rises, an increased percentage of inhibition is observed then ABTS radical scavenging increased with the extracts concentration. At the highest concentration (300 µg/mL), Ecs (21.29 ± 4.26%) and Uls (24.19 ± 0.47%) showed the highest inhibition. Vitamin C used as positive control has the best scavenging activity with a percentage of inhibition of 99.64 ± 0.75% at the same concentration.

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From Table 7 our results demonstrated higher presence of total phenol and flavonoid content in the *Emilia coccinea* stem (ECs) compared to the *Urena lobata* stem (Uls) extract which showed the highest content of flavonol. It is important to notice that these differences were significant in total polyphenol and flavonol but not between in flavonoid content in both extracts.

Our results presented in table 8 demonstrated the higher significant antioxidant capacity of *Emilia coccinea* stem (ECs) by FRAP assay compared to the *Urena lobata* stem (Uls) extract which showed the highest non significant antioxidant capacity by Phosphomolybdenum test.

According to table 9, IC_{50} of the all extracts is higher than Vitamin C for ABTS. The stem of *Emilia coccinea* showed an IC_{50} lower and upper than vitamin C respectively for OH and DPPH contrary to *Urena lobata* stem.

The correlations between the free radical scavenging properties using the Pearson's correlation analysis were also studied and the results are presented in the Table 10. All the

extracts demonstrated positive and significant correlation between ABTS and FRAP, OH and DPPH radicals and between Phosphomolybdenum and flavonoids with coefficient respectively of 0.860; 0.850 and 0.850 (Table 10). In the other way, negative significant ($p < 0.05$) correlations were found between flavonols and flavonoids on the one hand and phosphomolybdenum on the other hand with correlation of -0.850 and -0.865 respectively.

In fact, to overwhelm misunderstandings concerning the choice on the most effective antioxidative extract *in vitro* and also to help reporting the most reliable antioxidant activity order of extracts based on a statistical approach, principal component analysis (PCA) was applied to the antioxidant assays data. The total phenolic, flavonoid and flavonol content assays were not conducted on the pure molecules. Thus, factor analysis was performed on the data obtained only for plant extracts. A factor rotation using the Varimax method was performed for two factor loadings to see the correlations between assays that accounted for the total covariance of the plant extracts. In Figure 1, the variances caused by F1 and F2 were found 57.14% and 42.86% respectively. As can be found from the PCA graph, the results from OH, DPPH, FRAP, ABTS scavenging are respectively closely loaded to F1 while Phosphomolybdenum test, flavonoid and flavonol content assay are rather respectively closely loaded to F2 with 25 % of contribution each.

Both extracts have closed contribution to F1 showed as the best axis with 57.14 % of variance. Then contributions are respectively 36.881 and 36.352 % for Ul_s and Ec_s while vitamin C is rather closed to F2 with 52.71% of contribution.

DISCUSSION

In both extracts, MIC is lowered than MBC and MBC/MIC ratio of ECs is higher compared to the one obtained with Uls on *Staphylococcus aureus* strain. When the MBC of an antibiotic on a given strain is close to the MIC ($1 \leq MBC/MIC \leq 2$), the antibiotic is described as being bactericidal. On the other hand, when these values are relatively distant, ($4 \leq MBC/MIC \leq 16$), the antibiotic is known to be bacteriostatic. Lastly if the MBC/MIC >16, it is described tolerant. Then according to table 3, we can concluded than ECs is bacteriostatic and ULs, bactericidal.

These results with Gram negative bacteria could be due to the differences in the cell membrane of these bacterial groups. Indeed, the external membrane of Gram negative bacteria renders their surfaces highly hydrophilic [23], whereas the lipophilic ends of the lipoteichoic acids of the cell membrane of Gram positive bacteria may facilitate penetration

by hydrophobic compounds. According to [24], aromatic compounds group were known with their important antibacterial activity. The latter has been found to inhibit production of amylase and protease deteriorate cell wall, and cause cell lysis [25].

The mechanisms of antibacterial action of phenolic compounds are not yet fully deciphered but these compounds are known to involve many sites of action at the cellular level. Several authors explained this activity by the modification in permeability of cell membranes, the changes in various intracellular functions induced by hydrogen binding of the phenolic compounds to enzymes or by the modification of the cell wall rigidity with integrity losses due to different interactions with the cell membrane. Thus, the elevation of the lipophilic character of phenolic compounds enhances their antimicrobial activity by favoring their interaction with the cell membrane. This may induce irreversible damages of the cytoplasmic membrane and coagulation of the cell content that can even lead to the inhibition of intracellular enzymes. For example, condensed phenylpropanoids—tannins may induce damages at the cell membrane and even inactivate the metabolism by binding to enzymes while phenolic acids have been shown to disrupt membrane integrity, as they cause consequent leakage of essential intracellular constituents. Flavonoids may link to soluble proteins located outside the cells and with bacteria cell walls thus promoting the formation of complexes. Flavonoids also may act through inhibiting both energy metabolism and DNA synthesis thus affecting protein and RNA syntheses. In the case of Gram-positive bacteria, intracellular pH modification as well as interference with the energy (ATP) generating system were reported.

In our study this activity seemed to be influenced by phenolic composition and this is in accordance with previous studies that demonstrate a significant correlation between phenolic composition and antimicrobial activity. In fact [26] demonstrates that quercetin is an antibacterial molecule that can inhibit bacteria lipase production and inhibit d-alanine ligase activity which occurs in peptidoglycans production [27]. Other phenolic compounds are antibacterial such us rutin, (−)-epicatechin, and procyanidin B2 [28].

The phytochemical study of extracts revealed the presence of polyphenol compounds which have been suggested to decrease the oxidative stress in human especially through inhibition of the LDL-cholesterol oxidation [29]. Flavonoids found in the extract may inhibit the oxidative stress: -by scavenging free radicals by acting as reducing agent, hydrogen atom donating molecules or singlet oxygen quenchers; -by chelating metal ions; -sparing other antioxidants (e.g. carotene, vitamin C and E); and by preserving HDL associated serum paraoxonase

activity as described by Fuhrman and Aviram, [29]. Antioxidant properties of polyphenols are related to their chemical structure and depend on the number and arrangement of their phenolic hydroxyl groups. The amount of phenolics varies considerably from an extract to another. In fact, the polyphenol content in ECs extract is 13.45 times higher than the polyphenol content of ULs extract. This could justify the best ABTS and OH radical scavenging activities of this extract. In both extracts, several classes of polyphenols can also be identified including flavonol and flavonoid compounds.

In order to neutralize and fight against the deleterious effects of ROS, various antioxidant strategies have evolved either by increasing the endogenous antioxidant enzyme defenses or by enhancing the non- enzymatic through dietary or pharmacological means. The antiradical activities of various antioxidants were determined using the free radical: 2,2-Diphenyl-1 picrylhydraxyl ($DPPH^{\bullet}$) and 2,2'- azinobis 3-ethylbenzothiazoline-6-sulfonicacid ($ABTS^{\bullet+}$). The ABTS assay measures the relative ability of antioxidant to scavenge the $ABTS^{\bullet+}$ generated in aqueous and organic solvent systems while $DPPH^{\bullet}$ is a stable radical which loses its activity at 515 nm when reduced by an antioxidant or a free radical species. It is widely used to determine antiradicals/antioxidant activity of purified phenolic compounds as well as natural plants extracts [30, 31]. In the present research, after PCA analysis, *Urena lobata* stem extract has shown the best antiradical properties independently of the methods. This could be explained by the structure, number and arrangement of their phenolic hydroxyl group responsible for these activities. It is known that the antioxidant properties of many compounds are directly related to their reducing power and FRAP measures the ferric reducing ability of the antioxidant molecule. The analysis have showed that reducing power is 0.034±0.002 mg catechin equivalent/g of *Urena lobata* stem extracts and 0.20 ± 0.007 mg catechin equivalent/g of *Emilia coccinea* stem extracts. This could be due to synergetic effect of all of these chemical compounds found in different extracts.

The hydroxyl radical is formed *in vivo* by high energy irradiation leading to homolytic cleavage of water or from H_2O_2 in a metal catalyzed process. It can attract hydrogen atoms from biological molecules, including thiols leading to the formation of sulfur radicals capable of combining with oxygen to regenerate oxy-sulfur radicals which also damage biological molecules [32]. Endogenously, a free metal ion will react with H_2O_2 to produce the deadly free radical ($OH^{\bullet}$). Hence, scavenging of H_2O_2 and metal chelating processes are important for the cell [33]. The *Urena lobata* stem extract showed moderated

hydroxyl radical scavenging activity with IC_{50} of 4.488 mg of dry extract extracts being the strongest scavenger compared to vitamin C and *Emilia coccinea* stem extracts. This extract also showed moderated scavenging activity against $ABTS^{\bullet}$. Thus it can be used in reducing the effect of hydroxyl radical and $ABTS^{\bullet}$.

Our result might be due to the presence of phytochemical components, mainly polyphenolic compounds as flavonoids and flavonols as shown in table 9. *Urena lobata* has the highest and significant ($p < 0.05$) inhibitory potential for ABTS and OH, and *Emilia coccinea* extract, for DPPH. Plant acts as electron donors because of their content in phenolic compounds [34]. This may justify the radical scavenging activities power noted in the extracts tested. This result corroborates previous study which demonstrated that DPPH scavenging properties of plant extracts increase with the concentration of extracts [35, 36].

CONCLUSION

Based on the aforementioned results, we concluded that methanolic extracts of *Urena lobata* and *Emilia coccinea* stems have microbial and antioxidant potential activities *in vitro*. In spite the fact that the best scavenging activity differs from test to another, the effect was found to be significantly more effective with *Urena lobata* compared to *Emilia coccinea* extracts after PCA analysis. Therefore, these parts of plants could be used as good source of antioxidants and antibacterial for *Straphylococcus aureus*.

REFERENCES

1. Yang Y, Tsou CS, Lee TC, Wu WJ, Hanson PM, Kuo G, Engle LM, Lai PY. Distribution of 127 edible plant species for antioxidant activities by two assays. *J. Sc. Food Agric.* 2006; 86: 2395-2403.
2. Kaewpiboon C, Lirdprapamongko K, Srisomsap C, Winayanuwattikun P, Yongvanich T, Puwaprisirisan P, Svasti J, Assavalapsakul W. Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants. *BMC Compl. Altern. Med.* 2012; 12: 217.
3. Subhashini N, Thangathirupathi A, Lavanya N. Antioxidant activity of trigonella foenum graecum using various in vitro and Ex vivo models. *Int. J. Pharm. Sci.* 2011 (3): 96-102.
4. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Ann. Rev. Nutr.* 2001; 21: 386-406.

5. Aruoma OI. Nutrition and health aspects of free radicals and antioxidants. *Food Chem. Toxic*. 1994; l32: 671-683.
6. Naczk M, Shahidi F. Extraction and analysis of phenolic compounds in food. *J. Chromatogr. A* 2004; 1054: 95-111.
7. Papas AM. Beyond α-tocopherol: The role of the othertocopherols and tocotrienols, in Phytochemicals in Nutrition and Health, ed. by Meskin M.S., Bidlack W.R., Davies A.J. and Omaye S.T. CRC Press, New York, NY, 2000: 61-78.
8. Wu J, Wan Z, Yi J, Wu Y, Peng W, Wu J. Investigation of the extracts from Bidens pilosa Linn. var. radiate Sch. Bip. for antioxidant activities and cytotoxicity against human tumor cells. *J. Nat. Med*. 2013; 67: 17-26.
9. Dimo T, Nguelefack TB, Kamtchouing P, Dongo E, Rakotonirina A, Rakotonirina SV. Hyperotensive effects of a methanol extract of Bidens pilosa Linn on hypertensive rats. C R Acad Sci III 1999; 322: 323-329.
10. Kuan-Hung L, Hao Y, Su-Yi L, Chi-Ming Y, Hsiang-Ju T, Jia-Jen T, Pi-Yu C. Antioxidant Activities of Methanol Extracts from Selected Taiwanese Herbaceous Plants. *J. Food and Nutr. Res.* 2014; 2, no. 8: 435-442. doi: 10.12691/jfnr-2-8-2.
11. Sofowora EA. Phytochemical screening: Medicinal plant and traditional medicine in Africa. Spectrum Books Ltd. Ibadan. Nigeria. 1993: 270-289.
12. Harbone JB.. Phytochemical methods.A guide to modern techniques of plants analysis. Third edition ISSN: 0-412-5720-5 (HB) and 0-412-5727-0 (PB) 1998.
13. Hernandez NE, Tereschuk ML, Abdala LR. Antimicrobial activity of flavonoids in medicinal plants from Tafí del Valle (Tucumán, Argentina). J. Ethnopharmacol, 2000; 73: 317-322.
14. Clinical and Laboratory Standards Institute (CLSI), approved guideline. M44-A, 2005; 24: 12-28.
15. Clinical and Laboratory Standards Institute (CLSI), approved guideline, M27-A9, 2012; 32(9): 12-28.
16. Singleton VL, Orthofer R, Lamuela-Raventòs RM. Analysis of total phenols and other oxidation substrates and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. 1999; 299: 152-178.
17. Katalinié V, Milos M, Musi I, Boban M. Antioxidant effectiveness of selected wines in comparison with (+)-catechin. Food Chemistry. 2004; 86: 593-600.
18. Benzie IFF, Strain JJ.. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Analytical Biochemistry. 1996; 239: 70-76.

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19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans CA.. Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biology and Medicine. 1999; 26 (9-10): 1231-1237.

20. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Analytical Biochemistry. 1987; 165: 215-219.

21. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide. Radicals Food Chem. 1999; 64:555-9.

22. Kumaran A, Karunakaran R. In vitro antioxidant activities of methanol extracts of Phyllanthus species from India. Lebens-Wiss Technologie. 2007; 40:344–52.

23. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Letters in Applied Microbiology. 1998; 26 (2): 118–122.

24. Lis-Balchin M, Deans SG, Eaglesham E. Relationship between bioactivity and chemical composition of commercial essential oils. Flavour and Fragrance Journal. 1998; 13: 98–104.

25. Thoroski J, Blank G, Biliaderis C. Eugenol induced inhibition of extracellular enzyme production by Bacillus cereus. Journal of Food Protection. 1989; 52, (6): 399–403.

26. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005; 26(5): 343–356.

27. Wu D, Kong Y, Han C. d-Alanine:d-alanine ligase as a new target for the flavonoids quercetin and apigenin. International Journal of Antimicrobial Agents. 2008; 32(5): 421-426.

28. Zhang WY, Liu HQ, Xie KQ. gProcyanidin dimer B2 [epicatechin-(4..-8)-epicatechin] suppresses the expression of cyclooxygenase-2 in endotoxin-treated monocytic cells. Biochemical and Biophysical Research Communications. 2006; 345 (1): 508-515.

29. Fuhrman B, Aviram M. Flavonoids protect LDL from oxidation and attenuate atherosclerosis. Current Opinion in Lipidology. 2001; 12: 41-48.

30. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und- Technologie. 1995; 28: 25-30.

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31. Mahinda W, Shahidi F. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chemistry. 2000; 70: 17-26.

32. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. American Journal of Medicine. 1991; 91(3C): 14-22.

33. Gülcin I, Beydemir S, Alici HA, Elmastas M, Büyükokuroglu ME. *In vitro* antioxidant properties of morphine. Pharmacogical Research. 2004; 49: 59-66.

34. Duthie S, Dobson V. Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. Eur J Nutr. 1999; 38: 28–34.

35. Gul M, Ahmad F, Kondapi A, Qureshi I, Ghazi I. Antioxidant and antiproliferative activities of Abrus precatorius leaf extracts - an in vitro study. BMC Complement Alternat Med. 2013; 13(53).

36. Hilmi Y, Abushama M, Abdalgadir H, Khalid A, Khalid H. A study of antioxidant activity, enzymatic inhibition and in vitro toxicity of selected traditional sudanese plants with anti-diabetic potential. BMC Complement Alternat Med. 2014; 14(149).

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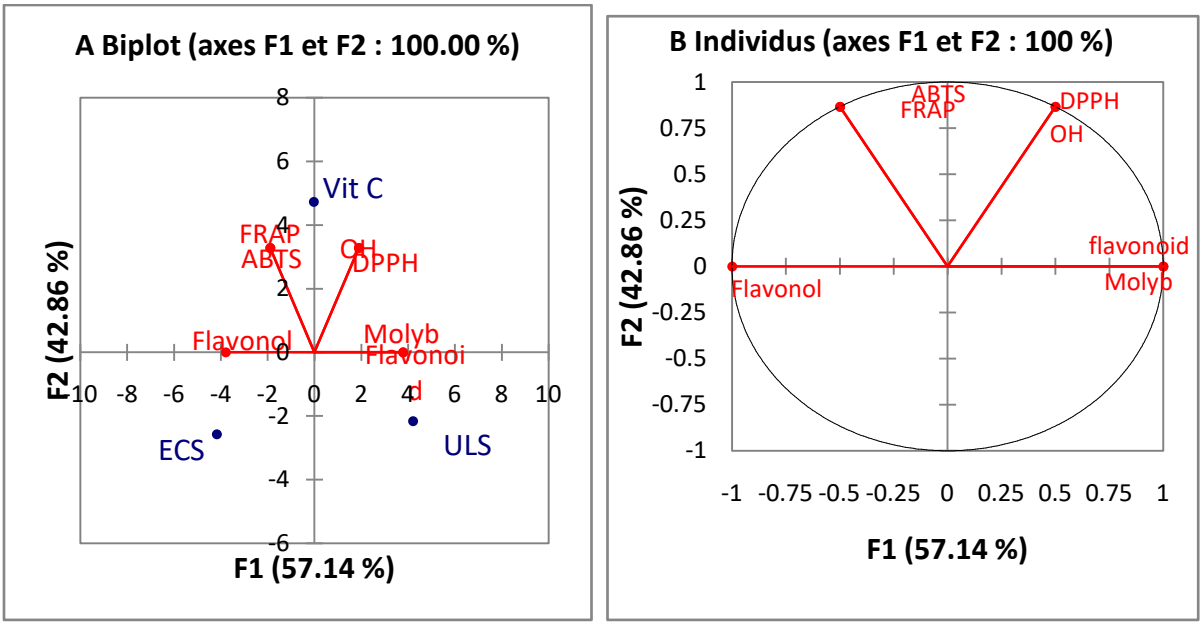

Ec$_s$: *Emilia coccinea* stem, Ul$_s$:*Urena lobata* stem. Molyb: Phosphomolybdenum test; Flavonol: Flavonol assay; Flavonoid: Flavonoid assay; ABTS: ABTS radical scavenging test; DPPH: DPPH radical scavenging test; OH: OH radical scavenging test. **A:** projection of the samples and tests around the F1 and F2 axis; **B:** distribution of the samples around the F1 and F2 axis.

Figure 1: Correlation between antioxidant capacity and free radical scavenging properties of the extracts.

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Table 1: Phytochemical screening of plant extracts.

Extracts	Phytochemical components						
	Phenol	Polyphenol	Tannin	Saponin	Flavonoid	Alkaloid	Stéroid and terpen
Ec_s	+	+	+	+	+	+	+
Ul_s	+	+	-	+	+	+	+

Ec_s: *Emilia coccinea* stem ; Ul_s: *Urena lobata* stem; (+): presence; (-): absence.

Tableau 2: Susceptibility test.

Extracts **Bacterial strains**	**ECs**	**ULs**	**Gen**
Proteus mirabilis	-	-	+
Escherichia coli	-	-	+++
Staphylococcus aureus	+	+	++
Shigella spp	-	-	++
Acinetobacter spp	-	-	++
Salmonella chaleresuis	-	-	++
Enterococcus faecalis	-	-	+++
Pseudomonas aeruginosa	-	+	+
Klebsiella ozaenae	-	-	++
Klebsiella pneumoniae	-	-	++
Enterobacter aerogenes	-	-	++

Ec_s: *Emilia coccinea* stem ; Ul_s: *Urena lobata* stem ; Gen *: Gentamicin;* (-): not active; (+): active.

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Tableau 3: Inhibition parameters: MIC, MBC, and MBC/MIC ratio.

Extracts / Reference	Inhibiton parameters (mg/ml)	Bacterial strains				
		S. aureus	*P.aeruginosa*	*E. faecalis*	*E. coli*	*P.mirabilis*
ECs	MIC	6.25	-	-	-	-
	MBC	25	-	-	-	-
	MBC/MIC	4	-	-	-	-
ULs	MIC	12.5	-	-	-	-
	MBC	25	-	-	-	-
	MBC/MIC	2	-	-	-	-
Gen (Reference)	MIC	6.25	6.25	6.25	6.25	6.25
	MBC	6.25	6.25	6.25	6.25	6.25
	MBC/MIC	1	1	1	1	1

Ec_s: *Emilia coccinea* stem; Ul_s: *Urena lobata* stem; Gen *: Gentamicin;* (-): not active; (+): active.

Tableau 4: DPPH scavenging potential (%) of the different plant extract at different concentrations.

Extracts	Concentrations' samples (µg/ml)				
	25	**50**	**100**	**150**	**300**
Ec_s	31.14 ± 6.51	21.98 ± 5.81	36.00 ± 3.22	49.16 ± 4.15	55.74 ± 8.67
Ul_s	35.45 ± 2.91	43.49 ± 9.46	47.66 ± 4.43	45.68 ± 3.12	52.31 ± 2.01
Vit C	97.47 ± 2.77	98.83 ± 0.32	98.74 ± 0.11	98.85 ± 0.14	98.38 ± 0.18

Values are given as mean ± SD and expressed as % of inhibition and mg equivalent catechin/g of dry weight. Catechin was used as standard. Ecs : *Emilia coccinea* stem; Ul_s :*Urena lobata* stem; Vit C : vitamine C

Tableau 5: Hydroxyl (OH) radical scavenging potential (%) of the different plant extracts at different concentrations.

Extracts	Concentrations' samples (µg/ml)				
	25	**50**	**100**	**150**	**300**
Ec_s	63.89 ± 4.81	63.89 ± 9.62	66.67 ± 6.67	67.22 ± 5.46	77.78 ± 4.81
Ul_s	16.67 ± 8.33	25.00 ± 8.33	61.11 ± 2.73	63.89 ± 2.73	80.56 ± 4.81
Vit C	44.44 ± 9.62	66.67 ± 8.33	77.78 ± 9.62	91.67 ± 14.43	100

Values are given as mean ± SD and expressed as % of inhibition and mg equivalent catechin/g of dry weight. Catechin was used as standard. Ecs : *Emilia coccinea* stem; Ul_s :*Urena lobata* stem; Vit C : vitamine C.

Tableau 6: ABTS radical scavenging potential (%) of the different plant extracts

Extracts	Concentrations' samples (µg/ml)				
	25	50	100	150	300
Ec_s	5.64 ± 0.16	6.96 ± 2.28	9.56 ± 1.26	15.18 ± 4.99	21.29 ± 4.26
Ul_s	7.53 ± 1.76	9.47 ± 1.94	15.49 ± 4.02	21.32 ± 2.69	24.19 ± 0.47
Vit C	19.57 ±6.49	36.52 ±9.26	83.66 ±3.81	98.49 ± 1.60	99.64 ± 0.75

Values are given as mean ± SD and expressed as % of inhibition and mg equivalent catechin/g of dry weight. Catechin was used as standard. Ecs : *Emilia coccinea* stem; Ul_s :*Urena lobata* stem; Vit C : vitamine C.

Tableau 7: Total phenol, flavonoids and flavonols contents of different plant extracts

Extracts	Total polyphenol *(CAE/g dried extract)*	Flavonoid *(QE/g dried extract)*	Flavonol *(QE/g dried extract)*
Ecs	151.33 ± 26.86	6.0 ±0.8	39.67±3.75
Uls	11.25 ± 0.25	5.2 ±0.1	49.33±5.77

Values are given as mean ± SD. Catechin and quercetin were used as standards. Ec_s : *Emilia coccinea* stem*;* Ul_s :*Urena lobata* stem.

Tableau 8: Antioxidant capacity of extract.

Extracts Compounds	**Ul_s**	**Ec_s**
FRAP	0.034 ±0.002	0.20 ±0.007
Phosphomolybdenum	0.22 ±0.01	0.17 ±0.03

Values are given as mean ± SD and expressed as % of inhibition of mg equivalent vitamin c /g of dry weight. Catechin was used as standard. Ec_s : *Emilia coccinea* stem; Ul_s :*Urena lobata* stem.

Table 9: Different values of IC_{50} of the plant extracts on the different radicals tested

	Tests		
Extracts	$ABTS^{\bullet+}$	$OH^{\bullet}$	$DPPH^{\bullet}$
Ecs	13.375	2.780	5.272
Uls	20.120	4.488	3.976
Vitamin C	11.194	3.424	4.697

Table 10: Results of the Pearson correlation of the different in vitro antioxidant assays

Variables	OH	ABTS	DPPH	FRAP	Phosphomolyb	Flavonoid	Flavonol
OH	**1**						
ABTS	0.500	**1**					
DPPH	**0.850***	0.500	**1**				
FRAP	0.500	**0.860***	0.500	**1**			
Phosphomolyb	0.500	-0.500	0.500	-0.500	**1**		
Flavonoid	0.500	-0.500	0.500	-0.500	**0.850***	**1**	
Flavonol	0.500	0.500	-0.500	0.500	**-0.865***	**-0.850***	**1**

**Values significantly different at alpha=0.050 (bilateral test). Phosphomolyb: Phosphomolybdenum test; Flavonol: Flavonols assay; Polyphenol: Polyphenol assay; Flavonoid: Flavonoids assay; OH: OH radical scavenging test; ABTS: ABTS radical scavenging test; DPPH: DPPH radical scavenging test; FRAP: ferric reducing antioxidant power test.*

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