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Research Article

Phytochemical Screening, Antimicrobial and Antioxidant Activities of *Aloe buettneri, Mitracarpus scaber* and *Hannoa undulata* used in Togolese Cosmetopoeia

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Abstract

Background: *Aloe buettneri*, *Mitracarpus scaber* and *Hannoa undulata* are three plants species used in the Togolese traditional medicine to cure dermatosis. This study aims at assessing their anti-oxidant and anti-microbial activities on acne-developing micro-organisms.

Methods: Six micro-organisms including *Cutibacterium acnes* ATCC 6919, *Pseudomonas aeruginosa* ATCC 27853; *Escherichia coli* ATCC 25922; *Klebsiella pneumoniae* ATCC 700603; *Staphylococcus aureus* ATCC 29213; and *Candida albicans* ATCC 35659 were used. Inhibition diameter was assessed using the agar well diffusion method. Minimum inhibitory and minimum microbicidal concentrations have been achieved through the liquid dilution method. Anti-oxidant activities were evaluated by DPPH antiradical scaving and FRAP methods. Phytochemical screening was also realized.

Results: All the microorganism's strains tested, excepted *Candida albicans and Escherichia coli,* were susceptible to plants extracts at 250 mg/mL in the agar well diffusion assay with inhibition diameters ranging from 12.10 ± 0.07 to 18.20 ± 0.10 mm. The MICs values were comprised between 15.625 mg/mL and 62.5 mg/mL, when MMCs ranged from 31.25 to 125 mg/mL. At the concentration of 500 µg/mL, the scavenging properties on DPPH radicals were 49.20 ± 0.15% for *H. undulata*, 41.29 ± 0.51% for *A. buettneri*, 59.57 ± 0.41% for *M. scaber* and 87.22 ± 0.03% for Quercetin. For FRAP assay, the effective concentration (EC_{50}) of *A. buettneri, M. scaber* and *H. undulata* extracts were 977.44 ± 1.13 µg/mL; 267.74 ± 10.13 µg/mL and, 272.54 ± 12.87 µg/mL respectively while quercetin presented the EC50 of 48.63 ± 2.00 µg/mL. The antimicrobial and antioxidant activities of these species might be required to the presence of polyphenols, tannins, flavonoids, triterpenes, saponoside and alkaloids identified by phytochemical screening.

Conclusion: The three plants extracts are all potential natural antimicrobial and antioxidant candidates for treating acne vulgaris.

Keywords: *Aloe buettneri, Mitracarpus scaber, Hannoa undulata*, antimicrobial activity, antioxidant activity, phytochemical screening, Acne vulgaris

Introduction

Acne vulgaris is a chronic inflammatory skin disease that affects 9.4% of the population in the world[1]. It is characterized by the development of comedo, papules, pustules, cysts, nodules and often scars. Many acne patients have dropped out of therapy due to the side effects and the failure of the treatments[2]. The pathophysiology of acne is associated with many factors: modifications of the pilosebaceous canal leading to excessive and altered sebum production, abnormal keratinization, micro-organisms, hereditary factors, inflammation, innate immune response and oxidative stress[3]. Some studies have established endocrinological and immunological, two essential factors which contribute to sebocyte differentiation and lipogenesis within the pilosebaceous unit which lead to the acne lesions[3]. In the recent years, it has been discovered that oxidative stress carries out a critical role in the acne pathogenesis[4 5]. *C. acnes* stimulates the production of superoxide anions in skin cells which initiate skin inflammation[6]. *C. acnes* is one of the main antibiotic-resistant micro-organisms responsible of acne lesions[7, 8]. There are several ways through which micro-organisms can lead disruption of the follicular epithelium and the inflammatory response. *C. acnes* promotes NLRP3-inflammasome via IL-1β release *in vitro* in monocyte-macrophages and in vivo in mice[9]. *C. acnes* also stimulates the expression of tumors necrosis factor-α (TNF-α) receptors, Toll-like receptors (TLRs), interferon (IFN), matrix

metalloproteinases and interleukins (IL-1, IL-8, IL-6, IL-12) by keratinocytes, leading to inflammation and hyperkeratinisation of the pilosebaceous tissue[10]. Current research has shown that the imbalance of the fascial skin micro-organism in acne vulgaris[11]. The skin microbiome, therefore, represent significant part in the development of acne[12, 13]. Plants extracts used to treat the skin diseases exhibited potent anti-microbial and antioxidant activities[14]. Aloe species have been well known for their medical and cosmetic purposes[15]. *Aloe buettneri*, *Mitracarpus scaber* and *Hannoa undulata* are used in the Togolese cosmetopoeia and to treat dermatosis. Their pharmacological studies have revealed some interesting properties that would help relieve acne sufferers: *Aloe buettneri* for its antioxidant and gastric antisecretory effects[16], anti-ulcer and anti-inflammatory effects[17,18]; *Mitracarpus scaber* for its antimicrobial and anti-radical effects[19], infected wound healing activities[20] and *Hannoa undulata* for also its antimicrobial activities[21]. In the present study, investigations were conducted on the *in vitro* anti-oxidant and anti-microbial activities on acne-induicing micro-organisms of *Aloe buettneri*, *Mitracarpus scaber* and *Hannoa undulata*.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Aloe buettneri (A. buettneri), Mitracarpus scaber (M. scaber)* and roots of *Hannoa undulata (H. undulata)* were harvested respectively in Lomé and Dapaong (Togo) during september 2019. The reference specimens were authenticated and stored in the Herbarium of the Laboratory of Botany and Plant Ecology of the University of Lome (Togo) under the numbers Togo15668, Togo15667 and Togo15669, respectively for *A. buettneri*, *M. scaber* and *H. undulata.*

Extraction

The collected samples were dried under air conditioning in the Physiology/Pharmacology Laboratory, University of Lomé (Togo). Both Roots and leaves were dried, then reduced to powder and a total of 300 g of each sample was macerated in 4 liters of ethanol-water mixture (5:5. v/v) within 72 hours. The filter as then subjected to vacuum evaporation at 40°C using a rotavapor (Buchi R- 210) and then lyophilized. The extraction yields have been determined according to the following equation:

Extraction yield (%) = (*W1* × 100)/*W2* where, *W1* is the weight of the extract obtained after evaporation and *W2* is the weight of the dried roots or leaves powder used.

Phytochemical analysis

Phytochemicals compounds such as polyphenols, tannins, flavonoids, sterols, triterpenes, saponosides, alkaloids, carbohydrates were detected in the extracts using the previous methods[22].

DPPH test

The colorimetric method was used to measure the scavenging capacity of the plant extracts[23]. The DPPH solution has been prepared in methanol and added to various concentrations of the extracts. The absorbance changes were determined at 517 nm after 30 minutes of incubation periods. Quercetin was used as a standard. These measures were evaluated in triplicate and the percentage of inhibition (Pi) has been calculated with the following equation: I= [(*A0–A1*) /*A0*] *100, where *A0* is the absorbance of the control, and *A1* is the absorbance of the extract or the standard.

Ferric reducing power

The antioxidant activity was evaluated by using also the colorimetric technique[24]. The FRAP reagent was obtained by adding 25mL of the acetate buffer (300 mmol/L); 2.5mL of 2,4,6-tripyridyl-triazine (TPTZ) solution (10 mmol/L) of Fe^{3+}-TPTZ in hydrochloric (40 mmol/L) and 2.5mL of aqueous iron chloride solution ($FeCl_3$-$6H_2O$) (20 mmol/L). One milliliter of quercetin or extract (62.5-500µg/mL) were allowed to react with 2mL of the FRAP solution and then left to incubate in the water bath at 37°C during 30 min. The readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The reference curve was constructed with Ferrous Sulphate (50-500 mmol/L) and the results were presented in mmol of Fe^{2+}.The results are compared with quercetin. The tests were carried out in triplicate.

Antimicrobial assays

Micro-organisms

Pseudomonas aeruginosa ATCC 27853(*P. aeruginosa*); *Escherichia coli* ATCC 25922 (*E. coli*); *Klebsiella pneumoniae* ATCC 700603 (*K. pneumoniae*); *Staphylococcus aureus* ATCC 29213 (*S. aureus*); and *Candida albicans* ATCC 35659(*C. albicans*) were taken from the strain collection of the bacteriology laboratory of the Togolese National Hygiène Institute. *Cutibacterium acnes* ATCC 6919 (*C. acnes*) was procured by "Laboratoires Humeau" (La Chapelle-sur-Erdre, France).

Presumptive testing

The germ susceptibility test for extracts was performed using the agar well diffusion method[25]. The relative susceptibility of acne-induised micro-organisms to the potential antimicrobial extract was assessed by a clear zone of growth inhibition around the well containing extracts. A fresh culture of the test microorganisms was grown on cooked blood agar for *C. acnes* or Mueller-Hinton agar for the rest of bacteria and sabouraud's chloramphenicol agar for *C. albicans* and was incubated 24h or 72 h at 37°C. About five colonies were removed into a tube containing sterile normal saline (0.9% NaCl) aseptically using a sterile metallic loop and calibrated to a turbidity of 0.5 MacFarland standards using a densitometer. The suspension was used to streak the surface of Muller Hinton agar plates with a sterile swab. A sterile 6 mm diameter reamer was used to make perforations in the fixed agar in the Petri dishes that contained the bacterial culture. Five (05) pits (three for the extract solution, one for positive control and another one for negative control) were realized on each plate. The pits were then filled with 50 µl of each extract at 250 mg/mL or gentamicin 10 µg/mL (appropriate control against S. aureus, *E. coli*, *P. aeruginosa* and *K. pneumonia*), clindamycin 100 µg/mL (control for *C. acnes*), and nystatin 250 mg/mL (control for *C. albicans*) were used as positive control. Dishes were incubated at 37°C during 72 h for *C. acnes*, 24 h for the remaining bacteria and at 25°C for *C. albicans*. All assays were performed in triplicate and the antimicrobial activities were expressed as the mean diameter of the inhibition zones (mm) produced by the plant extract.

Minimum inhibitory and minimum microbicidal concentrations

The minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC) have been assessed by the liquid dilution technique followed by spreading on an agar medium[26]. The Cooked blood agar was used for *C. acnes*, sabouraud's chloramphenicol agar was chosen for *C. albicans*, Muller-Hinton agar was used as the growth medium for the other bacterial strains. After adjusting

the inoculum to a 0.5 McFarland unit turbidity standard, microorganisms were grown with different extract concentrations ranging from 3.90625 to 125 mg/mL The microbial suspensions were incubated with the extract in Muller Hinton liquid medium at 37°C and then spread on the solid medium. The protocol consists in introducing 100 µl of the microbial suspension into 500 µl of the extract prepared in Muller Hinton. The controls were made up of extracts with no germs. The tests and controls were then streaked on the cooked blood agar for C. acnes, on Muller-Hinton agar for the other bacteria and on chloramphenicol sabouraud agar for C. albicans. All dishes were incubated at 37°C during 72 h for *C. acnes*, 24 h for the rest of the bacteria and 48 h for *C. albicans*. The non-existence of growth after incubation showed the susceptibility of the extract on the microorganism. The MIC is the lowest concentration of extract for which no growth was visually observed. The MMC is the highest dilution at which growth is absent or at 0.1% of the growth control.

Data analysis

The analyses were carried out in triplicate. Statistical data analysis was done using Graph Pad Prism 8.00 and Microsoft excel. The results are shown in percentages and means with standard error on the mean.

RESULTS

Extraction

Extraction yields obtained were 22.33%, 22% and 9.33%, respectively for leaves extracts of *A. buettneri* and *M. scaber,* and for roots extracts of *H. undulata*.

Phytochemical screening

The qualitative phytochemical analysis detected the presence of polyphenols, flavonoids, tannins, carbohydrates, triterpenes, and saponosides. Alkaloids and sterols were not detected in *M. scaber* leaves hydroethanolic extract. Sterols were absent in the roots hydroethanolic extract of *H. undulata*. The qualitative test results are shown in Table I.

Table I: Phytochemical screening

Metabolites	*A. buettneri* (LE)	*M. scaber* (LE)	*H. undulata* (RE)
Polyphenols	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Sterols	+	-	-
Triterpenes	+	+	+
Saponosides	+	+	+
Alkaloids	+	-	+
Carbohydrates	+	+	+

+: Presence; -: Absence; LE = Leaves ethanolic extract; RE = Roots ethanolic extract

DPPH assay

At the concentration of 500 µg/mL, the scavenging properties on DPPH radicals were 49.20 ± 0.15% for *H. undulata*, 41.29 ± 0.51% for *A. buettneri*, 59.57 ± 0.41% for *M. scaber* and 87.22 ± 0.03% for Quercetin (Fig. 1). *M. scaber* was the most active of the three plants extracts. The results of 2,2-diphenyl-1-pycrylhydrazyl (DPPH) radical scavenging assays showed that *Mitracarpus scaber* extract was the strongest antioxidant, followed by two extracts in the subsequent order: *Hannoa undulata* > *Aloe buettneri*

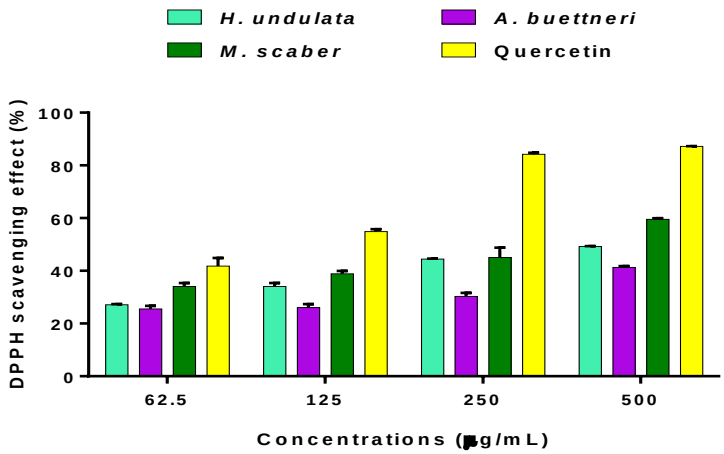

Figure 1: DPPH radical scavenging activity of plant extracts. The values are expressed as Means ± ESM, and correspond to percentage of DPPH radical inhibited, N = 3.

FRAP assay

The effective concentration (EC_{50}) of *A. buettneri, M. scaber* and *H. undulata* extracts were 977.44± 1.13 µg/mL; 267,74± 10,13 µg/mL and, 272.54± 12.87 µg/mL respectively while quercetin presented the EC50 of 48,63 ± 2,00 µg/mL. The results are shown in Fig. 2.

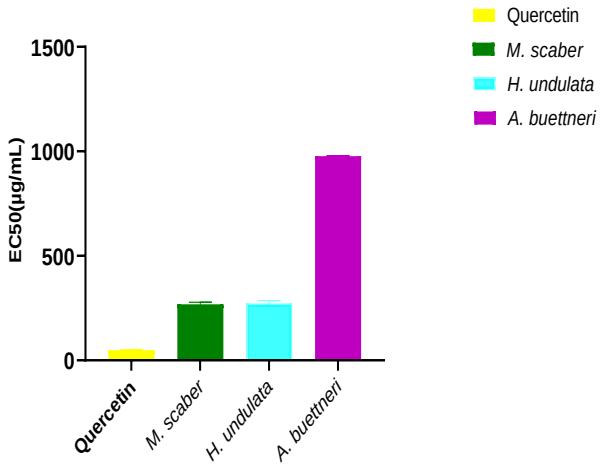

Figure 2: The effective concentration (EC_{50}) of plant extracts. The values are expressed as Means ± ESM, and correspond to percentage of DPPH radical inhibited, N = 3.

Antimicrobial assay

Presumptive testing

The inhibition of microorganism's growth has been materialized by clear zones around the well containing plants extracts. *A. buettneri*, *M. scaber* and *H. undulata* extracts were active on all tested microorganisms, excepted *C. albicans* and *E. coli,* with growth inhibition diameters ≥ 12 mm (millimeter). The zone of inhibition for extracts of the three plants resources was ranging from 12.22 ± 0.07 to 18.20 ± 0.10 mm (Table II). *A. b*uettneri was more active against *C. acnes* and *P. aeruginosa* with 18.20 ± 0.10 and 14.24 ± 0.17mm inhibition diameters. *M.scaber* was more active against *S. aureus* and *K. pneumonae* with 16.39 ± 0.07 and 14.79 ± 0.29 mm inhibition diameters respectively.*H. undulata* extract, compared with the two other extracts, showed a lower sensitivity on all germs tested. Comparatively, the zone of inhibition was 21.83 ± 0.40 mm for clindamycin against *C. acnes* and 26.77 ± 0.90 mm for nystatin against *C. albicans*. Gentamycin inhibition zones ranged from 22.19 ± 0.03 to 24.11 ± 0.93 mm. Gentamicin, Nystatin, Clindamycin gave the best diameters of inhibition compared with plant extracts.

Table II: Diameters of the inhibition zones of the extracts at 250 mg/mL and reference antibiotics for the different micro-organisms

	Plants extracts			Controls			
Microorganisms	*A. buettneri*	*M. scaber*	*H. undulata*	Gentamycin	Clindamycin	Nystatin	H_2O
C. acnes	18.20 ± 0.10	16.21 ± 0.04	15.24 ± 0.05	Ut	21.83 ± 0.04	Ut	0.00 ± 0.00
S. aureus	14.37 ± 0.23	16.39 ± 0.07	12.22 ± 0.06	24.11 ± 0.93	Ut	Ut	0.00 ± 0.00
K. pneumonae	13.71 ± 0.09	14.79 ± 0.29	13.29 ± 0.11	22.91 ± 0.87	Ut	Ut	0.00 ± 0.00
P. aeruginosa	14.24 ± 0.17	12.28 ± 0.45	12.10 ± 0.07	22.42 ± 0.08	Ut	Ut	0.00 ± 0.00
E. coli	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.19 ± 0.03	Ut	Ut	0.00 ± 0.00
C. albicans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Ut	Ut	26.77±0.09	0.00 ± 0.00

Ut = Untreated. H_2O = negative control. Units: millimeter. Each value corresponds to the mean (± S.E.M.) of three independent tests.

MINIMUM INHIBITORY AND MINIMAL MICROBICIDAL CONCENTRATIONS

The bacteriostatic and bactericidal effects of the extracts on the germs were also determined by the ratio of MMC/MIC ≤ 1 (Bactericidal); MMC/MIC ≥ 2 (Bacteriostatic). All extracts were bactericidal except extract of *A. buettneri* which was bacteriostatic *on K. pneumonae*. Results are summarized in Table III. The MICs values were in the range of 15.625 to 62.5 mg/mL and MMCs between 31.25 - 125 mg/mL. On *C. acnes*, *A. buettneri* gave the best effect with 31.25mg/mL for MMC; the MICs were the same for *A. Buettneri* and *M. scaber* (15.625mg/mL). But on *S. aureus* and *K. pneumonae*; *M. scaber* gave the best effect with 15.625mg/mL and 31.25 mg/mL respectively for MICs and MMCs. On *P. aeruginosa*, *H. undulata* gave the best effect with 31.25mg/mL and 62.5 mg/mL respectively for MICs and MMCs; *A. buettneri* and *M. scaber* gave the same effect with 62.5mg/mL and 125mg/mL respectively for MICs and MMCs.

Table III: MIC and MMC determination

	M. scaber (LE)			*A. buettneri* (LE)			*H. undulata* (RE)		
Bacterial strains	MIC	MMC	R	MIC	MMC	R	MIC	MMC	R
C. acnes	15.625	62.5	4	15.625	31.25	2	62.5	125	2
S. aureus	15.625	31.25	2	31.25	62.5	2	31.25	62.5	2
K. pneumonae	15.625	31.25	2	62.5	62.5	1	31.25	62.5	2
P. aeruginosa	62.5	125	2	62.5	125	2	31.25	62.5	2

MIC = Minimal Inhibitory Concentration. MMC = Minimal Microbicidal Concentration. MIC and MMC values are expressed in mg/mL. R represents the value of the ratio MMC/MIC. LE = Leaves ethanolic extracts; RE = Roots ethanolic extracts

DISCUSSION

The recent studies have suggested a contribution of oxidative stress to the pathophysiology of acne vulgaris[4, 5]. It has been shown that plants containing polyphenolic compounds can suppress free radicals[27] and may be beneficial for acne patients. For this purpose, the possible anti-oxidant activities of the three plant extracts were evaluated *in vitro* by the DPPH∗ free radical scavenger and ferric reducing anti-oxidant power assay. *M. scaber*, *A. buettneri* and *H. undulata* extracts showed anti-oxidant activities in a dose-responsive manner. Maintaining the balance between the rate of radical generation and the rate of radical scavenging would be essential for acne patients. The plants extracts have been shown their abilities to bind the DPPH∗ radical and also converted Fe^{3+} to Fe^{2+} that could help establishing the balance between the pro-oxidant and anti-oxidant system in acne vulgaris. These results provide confirmation of the anti-oxidant activities of *A. buettneri*[16], *M. scaber*[19] of previous studies. These findings suggest that plant extracts would prevent from *in vivo* oxidative skin cell damage and would be interesting in the cosmetic formulation purpose for some skin diseases in particular acne. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) which were the first line of defense against oxidative stress are diminished in the acne vulgaris[4, 5, 28]. Plant extracts would activate CAT, GPX and SOD in vivo to speed up effectively scavenging radical from epidermal cells in the pilosebaceous duct. The scavenging radical and the decrease of the reactive oxygen species by extract will be able to prevent oxidative stress and inflammation induced by *C. acnes*[6, 9, 10, 28]. These activities of plants extracts would be explained by the presence of the phytochemical groups in extracts especially polyphenolic compounds like quercetin[29]. The anti-oxidant potential of plants extracts is ascribable to the concentration of phenolics compounds. Polyphenolic compounds have the ability to release hydrogen or electrons and scavenger free radicals[30]. Future investigations will aim to study the effects of *M. scaber*, *A. buettneri* and *H. undulata* on the regulation of cellular mechanisms, to isolate and to identify phytochemical responsible for the anti-oxidant effects. The three Plants extracts could be good sources of natural anti-oxidants for acne patients.

Microorganisms are also one of the factors responsible for acne[12]. The current acne treatments are causing the emergence of resistant bacteria and serious side effects[31, 32]. Today, imbalance in the skin microbiome associated with an overgrowth of certain *C. acnes* phylotypes as well as other pathogenic microorganisms in the pilosebaceous unit. Restoring the skin micro-organisms, controlling the proliferation of *C. acnes* using antibiotics which do not induce resistance and have no adverse effects, and regulating sebum production are the main challenges of acne treatment. The discovery of alternative plants therapies against pathogenic strains while leaving commensal strains intact is promising and would be a real help in the treatment of acne. So, the antimicrobial activity of plant extracts on acne-causing bacteria and other micro-organisms found in acne lesions was investigated. The extracts of *A. buettneri, M. scaber, and H. undulata* have bacteriostatic and bactericidal effects on the tested micro-organisms except for *E. coli* and *C. albicans*. *A. buettneri* revealed the best activity on *C. acnes*; the main micro-organisms responsible for acne. *C. acnes*, an anaerobic pathogen, is implicated in the development of inflammatory acne[11]. *C. acnes* is implicated in the inflammation of acne by promoting the release of pro-inflammatory cytokines from the monocytes and leading to comedogenesis[11]. The plants extracts can effectively prevent *C. acnes*-induced skin inflammation by killing the micro-organisms and suppressing the microbial innate immune response like nitric oxide[33]. Extracts containing antibacterial substances capable of inhibiting *C. acnes* may also reduce the development of inflammatory acne. These extracts show broad-spectrum activities against the different susceptible microorganisms and would have some capacity to restore the skin microbiome because dysbiosis is also one of the causes of acne vulgaris[11]. The secondary metabolites contained in the different plant's extracts may act on their own or in synergy to inhibit or kill these micro-organisms. The literature had shown that polyphenolic compounds are also known to have antimicrobial properties[34, 35]. The results of the phytochemical test showed the presence of polyphenolic compounds, tannins and flavonoids in the extracts which would explain the anti-microbial activities. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to bind to bacterial cell walls. The mechanism of action of terpenes is not fully explained but is thought to be due to membrane damage by lipophilic compounds[36]. The anti-biotics such as erythromycin, clindamycin, gentamycin, nystatin used in this work as reference act on micro-organisms through several mechanisms[37]. Plants extracts may act on these mechanisms mainly by involving inhibition of the cell membrane, cell wall DNA synthesis, DNA gyrase, fatty acid synthesis, protein synthesis and RNA synthesis[37]. The active compounds in the extracts especially triterpenoids, flavonoids, tannins and saponins may have antibacterial activity[37].

The three plants extracts may potentially be used as the alternative treatment of the acnes and the antibiotic-resistant bacteria. The further investigations are needed to understand the anti-microbial mechanisms of the three extracts.

CONCLUSION

Extracts of *A. buettneri*, *M. scaber* and *H. undulata* have an anti-microbial effect on acne-causing micro-organisms and anti-oxidant properties. The findings of this research may explain the common use of these plants in the Togolese cosmetopeia and against dermatosis. *A. buettneri*, *M. scaber* and *H. undulata* will be good candidates to develop an alternative therapeutic for the treatment of acne vulgaris. However, the potential beneficial effect of the use of these plants in the treatment of *C. acnes* infection needs to be assessed *in vivo* in further investigations.

Conflict of interest

The authors have no reported conflicts of interest.

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