Journal of Applied Biosciences 167: 17280 – 17290
ISSN 1997-5902

The pharmacological effects of *Albizia gummifera* and *Spathodea campanulata* mixtures on *Staphylococcus aureus* and *Escherichia coli* species

Bayaga Hervé Narcisse[1], Guedje Nicole Marie[1], Ondoua Nguele Marc Olivier[2], Njinkio Nono Borgia Legrand[3], Fokunang charles[3], Ntsama Essomba Claudine[1*]
[1]Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O. Box 1364 Yaoundé - Cameroun
[2]Department of Pharmacology and Traditional Medicine, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O. Box 1364 Yaoundé - Cameroun
[3]Department of Pharmacotoxicology and Pharmacokinetic, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O. Box 1364 Yaoundé - Cameroun
***Corresponding author**: Ntsama Essomba Claudine**; Email**: ntsamaclaudine@yahoo.fr

Submitted on 3rd September 2021. Published online at www.m.elewa.org/journals/ on 30th November 2021
https://doi.org/10.35759/JABs.167.1

ABSTRACT
Objective: The aim was to determine the pharmacological effects from the mixture of *Albizia gummifera* and *Spathodea campanulata* trunk bark powder in proportions of 25/75, 50/50 and 75/25 (mass/mass) on the bacterial growth of two strains of *Staphylococcus aureus* and *Escherichia coli* species.
Methodology and Results: Primary and secondary metabolites such as carbohydrates proteins, polyphenols, cardiac glycosides and alkaloids were revealed , quantified in the aqueous and hydroethanolic extracts of these different preparations through colorimetric tests and spectrophotometric assays. The pharmacological effects resulting from the mixture of *Abizia gummifera* (A) and *Spathodea campanulata* (S) at different proportions was determined through the calculation and interpretation of the *Fractional Inhibitory Concentration Index* (FICI) parameter. A synergistic effect was observed in the aqueous extract of the mixture (A) and (S) in the respective proportions of 25/75 (FICI equal to 0.75 for *Escherichia coli* and 0.625 for *Staphylococcus aureus*). On the other hand, the antagonistic effect was manifested with the aqueous extract in proportions of 75/25 (FICI= 2.5 for *S. aureus*).
Conclusion and application of the results: These results suggest that the aqueous and hydroethanolic extracts of the mixtures of *Albizia gummifera and Spathodea campanulata* trunk barks possess secondary metabolites interacting with each other and have antibacterial activity towards the two strains of *Staphylococcus aureus* and *Escherichia coli* species. The pharmacological effects existing between these two plants vary with the proportions of each in the mixture. At 75/25, the mixture of *A. gummifera* and *S. campanulata* represents the original recipe used by the traditional healers, which may not be beneficial because of the antagonistic effects. Overall, this study underlines the importance of controlling the proportions of the different ingredients used in the preparation of effective herbal recipes.
Keywords: Pharmacodynamic interaction; *Albizia gummifera*; *Spathodea campanulata*; Antibacterial activity.

INTRODUCTION

The combination of drugs is a common practice. The aim is either to reduce the irritant or toxic effects of a component, to reinforce its activity, or to accelerate the curative effects of the various components of the drugs (Yi-ming Wang et *al.*, 2018). Many researchers claim that traditional therapists are not documented about the correct preparation and dosage of medicines, which for the most part are complex, and result from mixtures of several different plant ingredients (Noumi, 2010; Betti & Yemefa'a, 2011). Moreover, this form of combination in treatments or recipes usually presents some risks of interaction or toxicity in the human body (Yemoa et *al*., 2008). In a preliminary study by Bayaga et *al.* (2020), it was shown that the aqueous and hydroethanolic extracts of the traditional recipe consisting of mixing the trunk barks of *Albizia gummifera (J.F. Gmel.) C.A. Sm* and *Spathodea campanulata* P.Beauv in the respective proportions of 75/25 presented an antibacterial activity not significantly different from that observed with the aqueous and hydro-ethanolic extracts of each of the plants taken separately. Considering the results of this study, it appeared of great interest to quantitatively evaluate the phytochemical composition and the pharmacological effects resulting from the mixtures of trunk bark powder of *Albizia gummifera* (A) and *Spathodea campanulata* (S) on the bacterial growth of *Staphylococcus aureus* and *Escherichia coli* species.

MATERIALS AND METHODS

Plant material and microorganisms: The plant material and microorganisms were those used by Bayaga et *al*. (2020). They included trunk barks of *Spathodea. campanulata* P Beauv and *Albizia. gummifera* (J.F. Gmel.) C.A. Sm, collected at Akonolinga in the central region of Cameroon, as well as two bacterial strains (*Staphylococcus aureus* ATCC BAA-977 and *Escherichia coli* ATCC 25922), available at the Laboratory of Pharmacognosy and Pharmaceutical Chemistry of the Faculty of Medicine and Biomedical Sciences (FMSB) of the University of Yaoundé I -Cameroon.

Cell media used:

- Mueller Hinton agar (Liofilchem®) for the subculture of strains;
- Mueller Hinton Broth (MHB) Liofilchem® for the determination of Minimum Inhibitory Concentration (MIC).

Preparation of plant extracts: The preparation was based on study by Bayaga et *al*. (2020). After harvesting, the trunk barks of each plant were dried at room temperature in a rack for four weeks. Then, using an electric grinder (Blender, Warning Commercial, USA), the barks were pulverized into fine powder separately. These different powders were used for the preparation of aqueous and hydroethanolic extracts of *Albizia gummifera* (A) and *Spathodea campanulata* (S), as well as those of mixtures of these two plants in the respective proportions of 25/75, 50/50 and 75/25 (mass/mass).

Preparation of bacterial inoculants and stock solutions of extracts to be tested: These preparations were made according to the recommendations of the *Clinical and Laboratory Standards Institute* (CLSI, 2012). To this end, for each of the two selected microorganisms, a few bacterial colonies from a pure culture of 18-24 h on Mueller Hinton agar were introduced into 10 mL of physiological water to obtain an opacity equivalent to the 0.5 tube of the Mc Farland standard range (i.e. 1.5x108 cells/mL). The suspension was then diluted with MHB to 1/100th to obtain an inoculum with a concentration of 10^6 CFU/mL

Qualitative and quantitative phytochemical screening of metabolites: The main classes of bioactive compounds of the aqueous and hydro-ethanolic crude extracts of each plant as

well as of the different mixtures of these plants were measured by colorimetric tests. The quantification of some of these metabolites was done by spectrophotometric assays. The total polyphenol content of the extracts was determined using the method described by Singleton et *al.* (1965). The absorbance was read at 760 nm with a spectrophotometer. Quantification of flavonoids was carried out by a method adapted from Zhishen et *al.* (1999) with aluminium trichloride and soda. The absorbance of the pinkish colored solution was read at 510 nm against a blank. The aluminium trichloride $AlCl_3$ method (Kosalec et *al.*, 2004**)** with some modifications was used to quantify flavonols in the different extracts. The absorbance was read at 415 nm. Quantification of tannins was performed using the adapted method by Zhishen *et al.* (1999).

Determination of the Minimum Inhibitory Concentrations (MIC) and the nature of the effect resulting from the mixture of *A. gummifera* and *S. campanulata* in the different proportions: The different Minimum Inhibitory Concentrations (MICs) were determined by the macro-dilution method in liquid medium with visual appreciation of the growth of microorganisms as recommended by the *Clinical and Laboratory Standards Institute* (CLSI, 2012) and used by Bayaga et *al.* (2020). The possible interaction between *A. gummifera* and *S. campanulata was* assessed through the methodology used by Pei et *al*. (2009). This technique is based on the determination of the Fractional Inhibitory Concentration Index (FICI). FICI is equal to the total Fractional Inhibitory Concentration (FIC) of each plant present. The FIC of each plant was calculated by making the ratio between the Minimum Inhibitory Concentration (MIC) of each mixture of plants and the MIC of the concerned plant. The different formulas of calculation were the following:

$FICI = FIC_A + FIC_S$

FIC_A = MIC Mixture (A+S) / MIC A

FIC_S = MIC Mixture (A+S) / MIC S

With:

FIC_A = FIC *Albizia. gummifera* and FIC_S = FIC *Spathodea campanulata*

Interpretation:

- The effect will be qualified as synergistic for FICI values lower than 1;
- The antagonistic effect is observed for FICI values greater than 2;
- The addition effect for FICI values equal to 1;
- The indifferent for FICI values between 1 and 2.

RESULTS:

Phytochemical characterization of extracts: Table 1 presents the main groups of chemical compounds present in the different extracts. These are primary metabolites (proteins and carbohydrates) and secondary metabolites such as alkaloids, cardiac glycosides, polyphenols (flavonoids and tannins), and saponosides found in all the different extracts. Betacyans present in aqueous and hydroethanolic extracts of *Albizia gummifera* (A) were absent in all other extracts. The content of primary and secondary metabolites in the different parts of the plant is shown in Tables 2 and 3.

Table 1: Primary and secondary metabolites present in the different extracts of *Albizia gummifera* (A) and *Spathodea campanulata* (S).

	Reagents / Test	**Aqueous extracts**					**Hydroethanolic extracts**				
Metabolites		**A**	**S**	**A+ S (25/75)**	**A+ S (50/50)**	**A+ S (75/25)**	**A**	**S**	**A+ S (25/75)**	**A+ S (50/50)**	**A+ S (75/25)**
Lipids	NaOH	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
	Potassium dichromate	-	-	-	-	-	-	-	-	-	-
Carbohydrates	Molish	+	+	+	+	+	+	+	+	+	+
	Fehling	+	+	+	+	+	+	+	+	+	+
	Benedict	+	+	+	+	+	+	+	+	+	+
Protein	Biuret	+	+	+	+	+	+	+	+	+	+
	Millon Nasse	+	+	+	+	+	+	+	+	+	+
	Xantho protein	+	+	+	+	+	+	+	+	+	+
Alkaloids	Hager	+	+	+	+	+	+	+	+	+	+
	Wagner	+	+	+	+	+	+	+	+	+	+
	Mayer Waltz	+	+	+	+	+	+	+	+	+	+
	Tannic acid	+	+	+	+	+	+	+	+	+	+
Anthocyanins	Concentrated H_2SO_4	-	-	-	-	-	-	-	-	-	-
Chalcones		+	-	-	-	+	+	-	-	+	+
Betacyans	2N NaOH	+	-	-	-	-	+	-	-	-	-
Coumarins	$FeCl_3$ 10N	+	-	-	+	+	+	-	+	+	+
Flavonoids	Soda + Sulfuric acid	+	+	+	+	+	+	+	+	+	+
	Aluminum chloride	+	+	+	+	+	+	+	+	+	+
	Sulfuric acid	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	Killer killiani	+	+	+	+	+	+	+	+	+	+
Mucilage	Ethanol at 95°	-	-	-	-	-	-	-	-	-	-
Oxalates	Glacial ethanoic acids	-	-	-	-	-	-	-	-	-	-
Phobotannins		-	-	-	-	-	-	-	-	-	-
Total polyphenols	Lead acetate	+	+	+	+	+	+	+	+	+	+
	Iron perchloride	+	+	+	+	+	+	+	+	+	+

Quinones	Concentrated H_2S0_4	-	-	-	-	-	-	-	-	-	-
Resins	H_2S0_4	-	-	-	-	-	-	-	-	-	-
Saponosides	Foam test	+	+	+	+	+	+	+	+	+	+
Steroids	Libermann	-	-	-	-	-	-	-	-	-	-
	Salkowski	-	-	-	-	-	-	-	-	-	-
Total tannins	$CuSO_4$	+	+	+	+	+	+	+	+	+	+

Legend: (+) = presence of the desired metabolite. (-) = absence or trace presence of the desired metabolite.

Table 2: Metabolite content in aqueous extracts of *Albizia gummifera* (A) *and Spathodea campanulata* (S).

METABOLITES	**Aqueous extracts**				
	A	**B**	**A+B (25/75)**	**A+B (50/50)**	**A+B (75/25)**
Carbohydrates	34,98 ± 1,22	38,63 ± 2,11	34,98 ± 1,22	34,16 ± 0,70	46,76 ± 0,70
Total proteins (mg/ml BSA)	605,56 ± 19,25	616,67 ± 0,00	1027,78 ± 78,76	1200,00 ± 144,34	488,89 ± 19,25
Total tannins (mg Eq Tannic acid/g MS).	149,77 ± 3,72	160,52 ± 7,62	173,42 ± 5,18	162,13 ± 4,66	119,66 ± 2,79
Total flavonoids (mg Eq Quercetin /g MS).	60,98 ± 1,31	60,23 ± 13,82	73,86 ± 2,27	68,56 ± 3,47	42,80 ± 1,31
Polyphenols (mgEq Gallic acid/g DM).	3,82 ± 0,11	33,99 ± 5,70	51,96 ± 1,00	51,96 ± 1,36	29,41 ± 2,30
Total flavonols (mg Gallic acid eq/g DM).	373,06 ± 4,81	325,83 ± 36,32	270,28 ± 4,81	242,50 ± 0,00	242,50 ± 0,00

Table 3: Metabolite content in the different hydro ethanolic extracts of *Albizia gummifera* (A) *and Spathodea campanulata* (S).

METABOLITES	Hydroethanolic extracts				
	A	**B**	**A+B (25/75)**	**A+B (50/50)**	**A+B (75/25)**
Carbohydrates	30,91 ± 1,86	19,53 ± 0,70	39,45 ± 2,54	38,63 ± 0,00	47,98 ± 1,86
Total proteins (mg/ml BSA)	527,78 ± 9,62	577,78 ± 25,46	938,89 ± 151,23	655,56 ± 58,53	627,78 ± 143,69
Total tannins (mg Eq Tannic acid/g MS).	126,65 ± 6,11	114,82 ± 4,27	166,44 ± 11,29	139,02 ± 4,27	90,09 ± 0,93
Total flavonoids (mg Eq Quercetin /g MS).	32,95 ± 4,55	30,68 ± 0,00	74,62 ± 9,19	61,74 ± 1,31	26,14 ± 0,00
Polyphenols (mgEq Gallic acid/g DM).	30,72 ± 0,75	29,41 ± 1,51	47,71 ± 3,29	33,99 ± 1,31	22,55 ± 1,73
Total flavonols (mg Gallic acid eq/g DM).	186,94 ± 4,81	364,72 ± 17,35	331,39 ± 4,81	139,72 ± 4,81	139,72 ± 4,81

Minimum Inhibitory Concentration (MIC): Table 4 presents the MIC values of the different extracts against the tested bacterial strains. The different extracts inhibited the growth of all bacterial strains. MIC values between 3.125 and 12.5 mg/mL for the aqueous extracts and between 0.39 and 0.78 mg/mL for the hydroethanolic extracts were obtained against *S. aureus*; while those observed against *E. coli* were between 3.125 and 12.5 mg/mL for the aqueous extracts and between 6.25 and 12.5 mg/mL. The aqueous extract A+S (25/75) shows the highest antibacterial activity. On the other hand, the aqueous extract A+S (75/25) shows the lowest antibacterial activity with MIC values higher than 100mg/mL towards *E. coli*. This same extract presents a MIC value twice as high as that of the A+S aqueous extract (50/50) and 4 times higher than that of the A+S aqueous extract (25/75) towards *S. aureus*.

Table 4: Minimal inhibitory concentration of extracts against the tested bacterial strains.

Bacterial strains		***Escherichia coli***	***Staphylococcus aureus***
Types of Extracts		**MIC (mg/mL)**	**MIC (mg/mL)**
Aqueous extracts	A	12,5	12,5
	S	6,25	3,125
	A+S (25/75)	3,125	1,56
	A+S (50/50)	6,25	3,125
	A+S (75/25)	>100	6,25
Hydroethanolic extracts	A	12,5	0,78
	S	6,25	0,78
	A+S (25/75)	6,25	0,39
	A+S (50/50)	6,25	0,39
	A+S (75/25)	>100	0,78

Legend: A= *Albizia gummifera* and S= *Spathodea campanulata*

Nature of the effect resulting from mixing *Albizia gummifera* and *Spathodea campanulata* in the different proportions: Table 5 presents the different values obtained for the Fractional Inhibitory Concentration Index (FICI). A synergic action was observed only with the aqueous extract of the mixture of *Albizia gummifera* (A) and *Spathodea campanulata* (B) in the respective proportions of 25/75 with FICI values lower than 1 (FICI= 0.75 against *E. coli* and 0.625 against *S. aureus*), while an antagonistic effect was observed with the aqueous extract A+S (75/25). On the other hand, addition action was observed with the hydro-ethanolic extracts A+S (25/75) and A+S (50/50) towards the *S. aureus* strain (FICI= 1). Indifference action was observed with the extracts: aqueous A+S (50/50) towards the two bacterial strains, hydro-ethanolic A+S (25/75) and A+S (75/25) towards *E. coli* and with the hydro-ethanolic extract A+S (75/25) towards *S. aureus*.

Table 5: FICI values of the different extracts and nature of the effect resulting from the association of *A. gummifera and S. campanulata*.

Bacterial strains		***Escherichia coli***				***Staphylococcus aureus***			
Types of extracts		**FIC(A)**	**FIC(S)**	**FICI**	**Interpretation**	**FIC(A)**	**FIC(S)**	**FICI**	**Interpretation**
EA	A+S (25/75)	0,25	0,5	0,75	Synergy	0,125	0,5	0,625	Synergy
	A+S (50/50)	0,5	1	1,5	Indifference	0,25	1	1,25	Indifference
	A+S (75/25)	/	/	/	/	0,5	2	2,5	Antagonism
EHE	A+S (25/75)	0,5	1	1,5	Indifference	0,5	0,5	1	Additivity
	A+S (50/50)	0,5	1	1,5	Indifference	0,5	0,5	1	Additivity
	A+S (75/25)	/	/	/	/	1	1	2	Indifference

Legend: **A**= *A. gummifera;* **S**= *S. campanulata;* **EA** = Aqueous extracts; **EHE** = Hydroethanolic extracts.

DISCUSSION:

Qualitative and quantitative phytochemical screening: The different tests carried out revealed the presence of numerous secondary metabolites that may be responsible for the antibacterial activity. These are alkaloids, glycosides, polyphenols (flavonoids and tannins) and saponosides found in all extracts. The concentration of flavonoids presents in the aqueous and hydroethanolic extracts of *Spathodea campanulata* and *Albizia gummifera* species, were increased in the mixture of the two plants at proportions of 25/75 m/m (73.86 ± 2.27 mg Eq Quercetin /g MS for the aqueous extract and 74.62 ± 9.19 mg Eq Quercetin /g MS for the hydroethanolic extract). On the other hand, these flavonoid concentrations are decreased when the two plants were mixed at proportions of 75/25 (42.80 ± 1.31 mg Eq Quercetin /g DM for the aqueous extract and 26.14 ± 0.00 mg Eq Quercetin /g DM for the hydroethanolic extract) The same observation was found concerning the quantity of total tannins and total proteins. These results suggested a possible chemical reaction (beneficial or not) taking place between the different groups of compounds which eventually resulted in the modification of the initial structure of flavonoids, tannins, and proteins.

Antibacterial activity and nature of the interaction: The different extracts inhibited the growth of all tested bacterial strains. The presence in the different extracts of polyphenols (tannins, flavonoids), glycosides and alkaloids whose impact on the growth and metabolism of microorganisms is known, would justify this antibacterial activity (Atsafack et *al.*, 2016). Indeed, many authors suggested that the metabolites present in the different plant extracts might modify the permeability of the cytoplasmic membrane of bacteria (Stefanovick et *al*., 2012; Gebregergs et *al*., 2015). In the present study, the proportion of each plant in the mixture had an impact on the antibacterial activity. Indeed, various interactions in this case of: synergy, addition, indifference and antagonism were observed. The antagonistic effect was observed (FICI=2.5 > 2) with the aqueous extract of the mixture of *Albizia gummifera and Spathodea campanulata* in the respective proportions of 75/25. It should also be noted that according to the preliminary work of Bayaga et *al* (2020), this mixture was the original recipe used by traditional healers and for which the MIC values obtained against the bacterial strains of the species tested were the highest. Thus, although inhibition of bacterial growth was observed against *S. aureus*, the use of this mixture would not be beneficial given the resulting pharmacological effect (antagonism). From all the MIC values obtained with the different extracts, the lowest, between 3.25 and 1.56 mg/mL were observed on *E. coli* and *S. aureus*, respectively, with the aqueous extract of the mixture of *A. gummifera* and *S. campanulata* in the proportions of 25/75. The high content of this mixture in primary metabolites (reducing sugars and proteins) and secondary metabolites such as polyphenols (flavonoids and tannins) in comparison with the other extracts, would justify the halving of the MIC value initially obtained with the aqueous extract of *S. campanulata* towards the two bacterial inoculants tested. Moreover, the different values obtained of the Fractional Inhibitory Concentration Index (FICI= 0.75 and 0.625) respectively on *E. coli* and *S. aureus*, allowed to conclude the existence of a synergistic effect between these two plants (FICI< 1) when they were associated in 25/75 proportion. This result is in agreement with that of Fleurentin et *al.* (2011) who noted the fact that, mixture of plant extracts in which the association or synergy exists between their constituents is very often responsible for the desired effect. However, it would be important to pursue complementary studies in order to isolate the different molecules responsible for these

synergistic effects and to study the toxicity of these plant extracts.

CONCLUSIONS AND APPLICATION OF RESULTS

The nature of the pharmacological effect resulting from the mixture of *Albizia gummifera* and *Spathodea campanulata* in variable proportions was determined on two bacterial strains. The mixture of *A. gummifera* and *S. campanulata* in the proportions of 25/75 proved to be the best association or combination because of the synergistic action that was observed. In the proportions of 75/25, the mixture of *A. gummifera* and *S. campanulata* represents the original recipe used by the traditional healers, which may not be beneficial because of the antagonistic effects that result. Overall, this study underlines the importance of controlling the proportions of the different ingredients used in the preparation of effective herbal recipes.

ACKNOWLEDGEMENTS

Our thanks go to Mr. Ngobo Belinga Rémy, for sharing his knowledge and by indicating the initial recipe. We also thank the research teams of the Department of Pharmacognosy and Pharmaceutical Chemistry, as well as that of the Laboratory of Preclinical Studies and Toxicology of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I and Dr Dalle Ernest for the assistance they have shown us.

BIBLIOGRAPHY

Akharaiyi FC, Boboye B, Akpambang VO, Adetuyi FC, 2015. Phytochemical and Antioxidant Effect of *Spathodea campanulata* leaf Extracts. *International Journal of Biochemistry Research & Review*. 7(3): 148-59.

Atsafack SS, Kodjio N, Chegaing Fodouop SP, Ntemafack A, Kuiate JR, Gatsing D, 2016. *In vitro* Antisalmonellal and Antioxidant Activities of the Crude Extracts and Fractions from the Stem Bark of *Albizia gummifera* (J. F. Gmel.) C. A. Sm. British Journal of Pharmaceutical Research. 10 (6): 1-11.

Bayaga HN, Guedje NM, Tabi Omgba Y, Pola Yissibi E,Njinkio Nono BL, Assong Elombat DC, Fokunang C, Ntsama Essomba C, 2020. Pouvoir antibactérien des extraits aqueux et hydro-éthanolique du mélange d'écorces de tronc d'*Albizia gummifera* (J.F. Gmel.) C.A. Sm et *Spathodea campanulata* P.Beauv. *J. Appl. Biosci.* 154: 15881 - 15887

Betti JL & MebereYemefa'a SR, 2011. An ethnobotanical study of medicinal plants used in the Kalamaloué National Park, Cameroon. *Journ. Med Pl. Res*ch., 5 (8): 1447-1458.

Clinical and Laboratory Standards Institute (CLSI), 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. 32(2):88p.

Desalegn A, 2014. Antimicrobial Activity of Medicinal Plant Extracts and Their Synergistic Effect on Some Selected Pathogens. American Journal of Ethno medicine. 1(1):18-29.

Fleurentin J, Weniger B, Bourdy G, 2011. Traditions thérapeutiques et médicine de demain. Les enjeux de l'ethnopharmacologie.126p. ISBN 9782737352546.

Gebregergs T, Biniam T, Tadesse E, Alehegn W, 2015. *In vitro* Screening of Antibacterial Activities of Selected Ethiopian Medicinal Plants. *International Journal of Microbiological Research*. 6(1):27-33.

Kozalec I, Bakmaz M, Vladimir-Knezevic, 2004. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharm.* 54: 64-72.

Ntie-Kang F, LikowoLifongo L, Meva'aMbaze L, Nnange Ekwelle, Owono LC, Megnassan E, Judson PN, Wolfgang S, Efange SMN, 2013. Cameroonian medicinal plants: a bioactivity versus ethnobotanical survey and chemotaxonomic classification. *BMC Complementary and Alternative Medicine*. 13(147):18p.

Noumi E., 2010. Ethno medicines used for treatment of prostatic disease in Foumban, Cameroon. *Afr. & Journ.Pharm*, 4 (11): 793-805.

Pei RS, Zhou F; Ji BP, Xu J, 2009. Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against *E. coli* with an improved Method. Journal of Food Sciences. 74: 379–83.

Rajesh Kowti, Harsha R, Mohammed GA, Hareesh AR, Thammanna Gowda SS, Dinesha R, Satish Kumar BP, Irfan Ali M, 2010. Antimicrobial activity of leaf and flower of *Spathodea campanulata* P. Beauv. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 1(3):8.

Singleton VL and Rossi JA, 1965.Colorimétrie des composes phénoliques totaux avec les réactifs acide phosphomolybdique-phosphotungstique. *Am J Enol Vitic*.144-158.

Stefanovic OD, Stanojevic DD, Comic LR, 2021. Synergistic antibacterial activity of salvia officinalis and cichorium intybus extracts and antibiotics. *Act Pol Pharm D Res*, 69: 457-463. ISSN 0001-6837.

Yemoa AL, Gbenou JD, Johnson RC, Djego JG, Zinsou C, Moudachirou M, 2008. Identification et étude phytochimique de plantes utilisées dans le traitement traditionnel de l'ulcère de Buruli au Bénin. Ethnopharmacologia. 42:48-55.

Yi-ming Wang, Ling-cong Kong, Jie Liu and Hong-Xia Ma, 2018. Synergistic effect of eugenol with Colistin against clinical isolated Colistin-resistant *Escherichia coli* strains. Antimicrobial resistance and Infection Control.7:17. DOI 10.1186/s13756-018-0303-7.

Zhishen J, Mengcheng T, Jianming W, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *F Chem.* 64:555-559.