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Phytochemistry and evaluation of the antimicrobial synergistic effects of the different organs of *Magifera indica*

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***ABSTRACT**: In a context of limited resources and research into natural remedies, the different organs of Mangifera indica are among the natural substances used for the treatment of respiratory infections. In the literature, the antimicrobial activity of these organs has been described separately, but the synergistic effects between them remains unevaluated. This work aims to identify the possible synergistic effects that may exist between the leaves, steam barks and seeds of Mangifera indica. In this context, ethanolic extracts of different organs of Mangifera indica and their mixtures were carried out. Qualitative and quantitative phytochemical analyses and the antimicrobial activity on seven strains of microbes were determined. Among the different extracts, six strains (gram-positive and negative) of bacteria out of seven were sensitive to the extracts of the seeds and the mixture (seeds/steam barks) with inhibition diameters going up to 21.5 mm. Minimum inhibitory concentrations (MIC) between 5 and 20 mg/ml were noted. The lowest values are recorded at the level of seeds and the mixture (seeds/steam barks). The same fractions indicated the lowest minimum bactericidal concentration (MBC) (25 mg/ml). The phytochemical study of the most effective fractions indicated the common presence of families of compounds such as Reducing compound, Alkaloids, Flavonoids, Catechy tannins, Gallic tannins, Saponin, Terpenoids, Mucilages, O-heterosides. between all organs of the plant, seeds were more effective. A synergistic effect was observed by mixing seeds and steam barks. Mangifera indica seeds remain very promising substances in the search for new anti-infectious phyto-drugs.*

***KEYWORDS:** Mangifera indica's organs, synergistic effects, phytochemical analyses, antimicrobial activity.*

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I. INTRODUCTION

The respiratory infections are among the leading infectious causes of morbidity and mortality in the world. According to WHO, they are the third leading cause of death in world [1-3]. They therefore pose a serious public health problem, especially in developing countries. The case of Africa is even more alarming due to the climate, their frequency and their economic impact [4,5]. Respiratory infections can be caused by various pathogens such as viruses, bacteria, pathogens and other microorganisms, which are spread by direct contact, through the air or through contaminated surfaces. Pneumococcal pneumonia has been reported to be more deadly than malaria, measles and AIDS combined while only 30% of patients have access to medical treatment [6]. In addition to financial problems, the current crisis of antibiotic resistance poses a serious threat to public health [7,8]. This resistance reduces the effectiveness of modern drug treatments. In this context, the advent of new cheaper, natural and less toxic antibiotics is a great alternative. A wide range of bioactive secondary metabolites generated by medicinal plants is the subject of several studies and aims to develop new antibacterial agents effective against microorganisms. Many plants of the African flora have shown very interesting antibacterial properties [9-13]. Mango tree is one of the many edible plants that has many pharmacological effects. Several uses are attributed to the plant in traditional medicine. The different organs of the plant (leaves, steam bark, roots and seeds) are exploited in the treatment of syphilis, parasitic diseases, worms, inflammation, cough, hiccups, hyperdipsia, burning sensation, hemorrhage, hemoptysis, hemorrhoids, diarrhea, dysentery, wounds, ulcers, anorexia and dyspepsia [14-21]. Through these different works, various activities related to each organ of the plant have been described, but the comparative antimicrobial effect of the three organs (leaves,

steam bark and seeds) of the plant remains unreported. In addition, it would be interesting to identify any possible synergistic effects that exists between these three organs. This work therefore aims to compare the antimicrobial activity of the different parts of *Mangifera indica* and to identify possible synergistic effects that exist between them.

II. MATERIALS AND METHOD

Extract Preparation

In some studies, the ethanolic fraction had been shown to be more active. In addition, ethanol is a very little toxic solvent and according to some authors allows to extract the maximum of chemical compounds [14,22]. That is what motivated us to take an interest in the ethanolic extract of the seven fractions. The plant material used in this study consists of the different organs of *Mangifera indica* (leaves, steam bark and seeds). The different organs were carefully washed, cut and then left to dry in an oven at 35 degrees for 72 hours. The three dried substances were carefully crushed separately using an electric grinder. The crushed material is sieved and then packaged in glass jars protected from humidity for the rest of the work [14].

Preparation of the combinations

Various respective combinations of the different organs of *Mangifera indica* were made. The overall mass of each combination is equal to 60 g. They were made in the following proportions:

L60 (60g leaves; 00g steam bark; 00g seeds); **B60** (00g leaves; 60g steam bark; 00g seeds); **S60** (00; 00; 60) (00g leaves; 00g steam bark; 60g seeds); **L30/B30** (30g leaves; 30g steam bark; 00g seeds); **L30/S30** (30g leaves; 00g steam bark; 30g seeds); **B30/S30** (00g leaves; 30g steam bark; 30g seeds);

L20/B20/S20 (20g leaves; 20g steam bark; 20g seeds).

The principle adopted during the constitution of the mixtures is based on the combination of the organs two by two in equal proportions and then the combination of the three organs in the same proportions. This led to the formation of seven combinations. 60 g of powder of each combination were crushed and recovered in 600 ml of ethanol 96°C for 72 hours. After agitation and homogenization, the mixture is filtered on Wathman paper and the filter is concentrated in a rotary evaporator at a temperature between 55°C and 60°C with the help of vacuum pump to obtain the extract. Drying was finalized in an oven at 35°C for 72 hours. The dry extract obtained was stored in a refrigerator at 4°C [14].

Antimicrobial activity assessment methods

Some Bacteria responsible for respiratory infections were identified to serve as animal material during this study. These are: *Klebsiella pneumoniae, Clinical Staphylococcus aureus, Acinetobacter baumannii, Staphylococcus aureus Reference, Escherichia coli, Pseudomonas aeruginosa, Streptococcus oralis.*

Sensitivity test

It was done according to the disc method inspired from the one described by Bauer et al. (1996) [23]. Brieflt, 1 ml of pre-culture of 18-24 h (10^6 UFC/ml) enabled planting a box of Petri dishes containing agar Mueller Hinton by flood. After seeding, the sterile Whatman paper discs (5 mm de diameter) were deposited with sterile pince. These discs have been carefully impregnated with 30 µl of plant extract (20 mg/ml. The dishes were kept for 15-30 min at room temperature before incubation at 37°C. The inhibition zones diameters were measured after 24 to 48 hours using a ruler graduated [24]. For each extract, the experiment was performed induplicate.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC has been determined by macrodilution method with Visual assessment of the growth of microorganisms [25]. Briefly, nine concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 µg/ml) was performed in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (106 UFC/ml). After 24 h of incubation turbidity tubes was examined relative to the control tube containing distilled water and the inoculum (10^6 UFC/ml).

Determination of the Minimum Bactericidal Concentration (MBC)

The MBC was determined by solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37 ° C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC [26].

Preliminary phytochemical screening of S60 and B30/S30 Factions

Qualitative phytochemical screening

The qualitative phytochemical screening was performed based on colouring or precipitation reactions. It is made directly on the ethanolic extract of S60 and B30/S30 fractions according to Houghton and Raman method (1998) [27]. Quantitative phytochemical tests were carried out according to the method of Harbon (1984) and Umeaku et al (2018) [28,29].

Quantitative phytochemical screening of S60 and **B30/S30 Factions**

Estimation of total flavonoid content

Flavonoid content of extract was determined by colorimetric method described by Chang et al. (2002) [30]. The plant extract was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8ml of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using spectrophotometer. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of rutin/gm of extract).

Estimation of total tannins

The total tannin content in the plant extract was determined by Folin-Deins reagent method adopted by Polshettiwar et al. (2007) [31]. The absorbance was measured at 755 nm.

Estimation of total saponins

Twenty grams of plant powder was dispersed in 200 ml of 20% ethanol. The suspension was heated over a water bath for 4 h with stirring at about 55 °C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40ml over water bath at about 90 °C. Twenty ml of diethyl ether was added to the concentrate and shaken vigorous. The aqueous layer was recovered while the ether layer was discarded.

The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was evaporated. Then, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage according to Okwu and Ukanwa (2007) determination [32].

Estimation of total alkaloids (gravimetric method)

Ten grams of the plant powders was extracted with 90 % ethanol till exhaustion tested with Mayer's reagent using the standard procedure described by Woo & Püls (1977) [33].

Estimation of total phenolic content

Determination of total phenolics in plant extract was determined by using modified Folin-Ciocalteu method (Maurya and Singh, 2010) [34,35]. Gallic acid solution (sigma chemical) was used as a standard and prepared in various concentration 2-10 µg/ml to be a standard curve. Concentration of 1mg/ml of plant extract was also prepared and 0.5ml of each sample were introduced into test and mixed with 2.5 ml of a 10 fold dilute Folin- Ciocalteu reagent and 2 ml 0f 7.5 % sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30min at room temperature before the absorbance was read at 760 nm spectrophotometrically. All determination was performed in triplicate. Determination of total phenol content in the extracts were calculated using the linear regression equation of the calibration curve and expressed as gallic acid equivalent per gram of extract.

Carbohydrates content

Total, soluble and insoluble carbohydrates were estimated by using the method described by Chaplin and Kennedy (1994) [35]. Total carbohydrates were extracted by dissolving 1g of powder in 2-5 ml of 2M HCl in a sealed tube. The sealed tube was heated at 100 ºC for a period of 2-5 h. The extracted sugars were estimated using the general phenol- sulfuric acid assay. The absorbency was measured at 490 nm after 30 min.

For soluble carbohydrates half gram plant powders were extracted with ethanol/water (80% v/v) by reflux for 2 h. The alcohol was removed from the alcoholic extract by evaporation under reduced pressure. The aqueous extract was clarified using Carrez reagent, and then its volume was completed to 100 ml with dist. water. Then calculate insoluble carbohydrates = Total carbohydrates - soluble carbohydrates.

Data treatment and analysis:

The spreadsheet Microsoft Excel version 2013 has been used for the capture and encoding the data.

III. RESULTS AND DISCUSSION

Extraction

A total of seven extracts were obtained with yields ranging from 9.167% to 15.66% (Table 1). The lowest yields were noted with seed of *mangifera indica*. The highest yield is obtained with The Stem bark. The optimum yields are obtained with the leaves. The different mixtures gave intermediate yields. All substances have a pasty appearance with traces of oil accentuated in the seed extracts (Table 1).

Table 1. Extraction yields, codes and physical appearance of the extracts

Extracts	**Extract Codes**	**physical appearance**	**Extraction yields (%)**
M- leaves (60g)	**L60**	Pâte	14,16 ± 0.22
I-Stem bark (60g)	**B60**	Pâte	15, 66 ± 0.45
K- Seeds (60g)	**S60**	Pâte	9,16 ± 0.25
N- leaves (30g), Stem bark (30g)	**L30/B30**	Pâte	14,16 ± 0.08
H- leaves (30g), Seeds (30g),	**L30/S30**	Pâte	13,33 ± 0.88
J- Stem bark (30g), Amande (30g)	**B30/S30**	Pâte	12,5 ± 0.85
L- Stem bark (20g), Seeds (20), leaves (20g)	**L20/B20/S20**	Pâte	15,83 ± 0.34

Leaves and steam barks contain more fiber than seeds. This facilitated filtration at these organs. Filtration was slower with seeds. Mango seeds would contain less fiber than leaves and steam barks. The mixture carried out with the other organs of the plant was beneficial because an increase in extraction yield was noted in the combinations containing seeds. The low yield noted with seeds can also be explained by the fact that they would contain enough apolar compounds that were not completely extracted by ethanol. This can also explain the very oily pasty appearance noted in this extract. Since the extraction solvent is polar, it could not extract all the lipophilic compounds from the seeds. We know that oils are generally composed of very non-polar compounds. Authors have also successfully extracted and studied the oils contained in these seeds [36]. These different extracts obtained are evaluated on seven strains of bacteria (gram-positive and negative) in order to determine the inhibition diameters, the minimum inhibitory and bactericidal concentrations.

Extracts inhibitory diameter zone with the reference strains

Figure 1 indicates the inhibition diameter of the seven fractions on the seven strains of microbes. The values of the inhibition diameters obtained are between 8.5 mm and 21.5 mm. The largest inhibition diameter of 21.5 mm was noted with the combination B30/S30 on the strain *A. baumannis*. The smallest diameter is always noted with the same combination on the strain *S. aureus* CL. A comparative study of the start substances taken individually indicates that the seeds are more active than the other organs of the plant. They inhibited six germs out of seven in total, with a percentage of 85.71%. This activity is followed by that of the steam barks. The steam barks inhibited five germs out of seven with a percentage of 71.42%. The lowest activity was noted in mango leaves which inhibited only one germ out of a total of seven with a percentage of 14.28%.

When considering the mixtures, the mixture of bark and nuts (B30/S30) was the most active (inhibited 85.71% of the microbes) with the largest inhibition diameters. The combination of leaves and nuts (L30/S30) inhibited three germs out of seven with a percentage of 42.86%. The combinations that had low activities were those of leaves with bark (L30/B30) and those composed of the three organs (L20/B20/S20) with a percentage of 28.57%. In general, among all the combinations, those containing mango nuts were the most active. Those containing leaves were the least active. Different effects appeared; the synergistic effect and the antagonistic effect. When the B30/S30 mixture is taken, the observed inhibition diameters are larger than the inhibition diameters of the nuts and the barks. In this case, a synergistic effect is clearly noted. When the L30/S30, L30/B30 and L20/B20/S20 mixtures are considered, the initial effect of the nuts and the barks has decreased. An antagonistic effect is noted. Even if a combination must be made, it must be made in well-defined proportions, otherwise an antagonistic effect will appear.

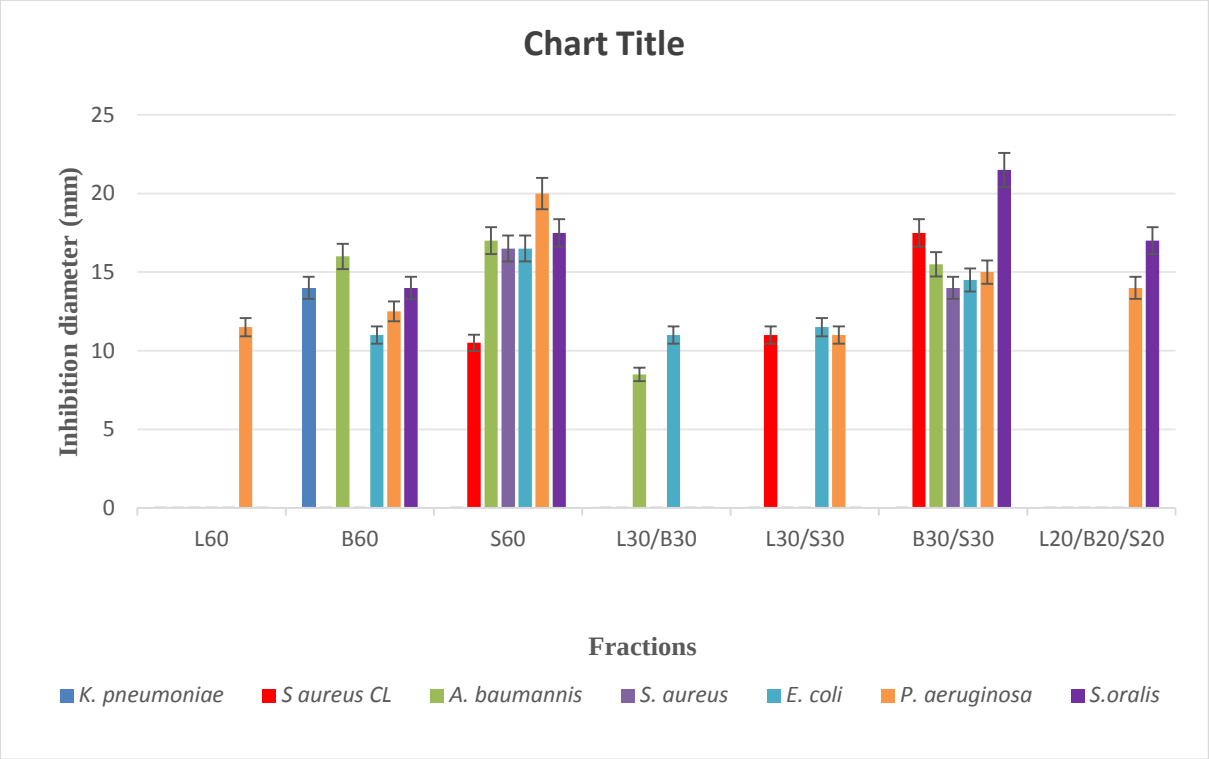

Figure 1. Diameters of inhibition of different fractions

In order to identify the fraction that inhibits more germs and presents the highest diameters of inhibition, the average diameters of inhibition taking into account all the strains used were evaluated and led to Figure 2.

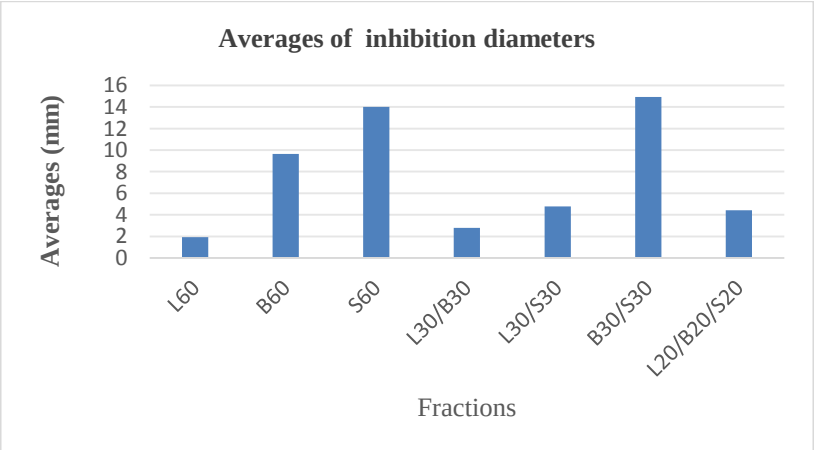

Figure 2: Average inhibition diameters of the different combinations of extracts

By reading this diagram, it appears that the combination B30/S30, S60 and B60 have the largest averages inhibition diameter. The respective average values of these diameters are 14.93 mm, 14 mm, and 9.63 mm. The combination B30/S30 remains the most active. It inhibited more germs with very good values of inhibition diameters. This diagram confirms the good activity of the three substances and the synergistic effect that exists between the steam barks and the seeds. These values are largely higher than the values found during our previous work with other substances. The highest average found with these substances was about 7 mm [37]. Following the determination of the inhibition diameters, to confirm the antimicrobial activities noted above, the minimum inhibitory and bactericidal concentrations were also evaluated.

Minimum Inhibitory Concentrations (MIC) of extracts

All extracts inhibited at least one strain. The extracts inhibited the proliferation of most pathogenic bacteria with variable minimum inhibitory concentrations (MIC). Reading the results, the minimum inhibitory

concentrations have values between 5 and 20 mg/ml (Figure 3). Of the three substances taken individually, the bacteria were sensitive to the steam barks and seeds. Six out of seven were sensitive to the seed extracts. Five out of seven germs were sensitive to the steam barks. The seeds therefore remain the most effective organs of the three studied. This result confirms those obtained in our previous work [14]. Of all the mixtures, B30/S30 is always the most active. Six out of seven germs were inhibited with fairly low MIC values. The mixture of leaves and seeds had good values especially on *S. aureus* CL (5 mg/ml) but only inhibited three germs out of the seven studied (42.85%).

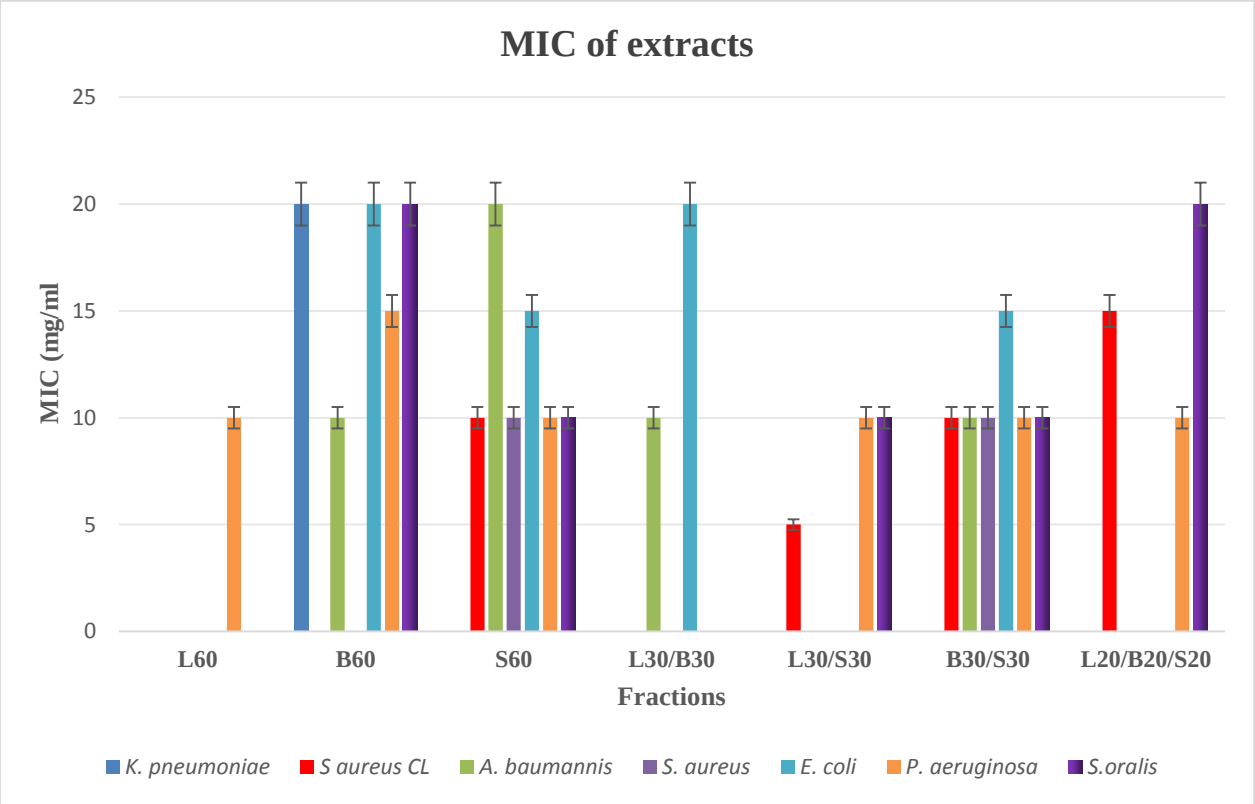

Figure 3: Minimum inhibitory concentrations (mg/ml) of the extracts

A good correlation is noted between the inhibition diameters and the minimum inhibitory concentrations. The B30/S30 mixture indicated the smallest MIC values and the largest inhibition diameters on most of the strains used. The notion of synergy is again confirmed at the level of this mixture. All the mixtures containing the leaves experienced a decrease in their effect. The antagonistic effect therefore clearly appears with the fractions L30/S30, L30/B30 and L20/B20/S20. The seeds and steam barks taken individually inhibit 86.71% of the strains while their mixture with the leaves leads to a decrease in activity up to a percentage of 28.57%. It emerges from this paragraph that the mixtures of the different organs of a plant do not systematically lead to obtaining a synergistic effect. This work clearly highlights the existence of agonist and antagonist effects that can exist between the organs of *mangifera indica*. Some authors have also noted in their work that synergistic or antagonistic effects can occur during the preparation of mixtures [38].

Minimum Bactericidal Concentration (MBC) (mg/ml) of extracts

Figure 4 shows the minimum bactericidal concentrations (MBC). In addition to inhibiting the strains used in this work, the extracts also showed a bactericidal effect. The concentrations that can kill germs are between 25 and 50 mg/ml. Concentrations above 50 mg/ml have not been mentioned in this figure. The best bactericidal activities were also obtained with the fraction S60 (figure 4).

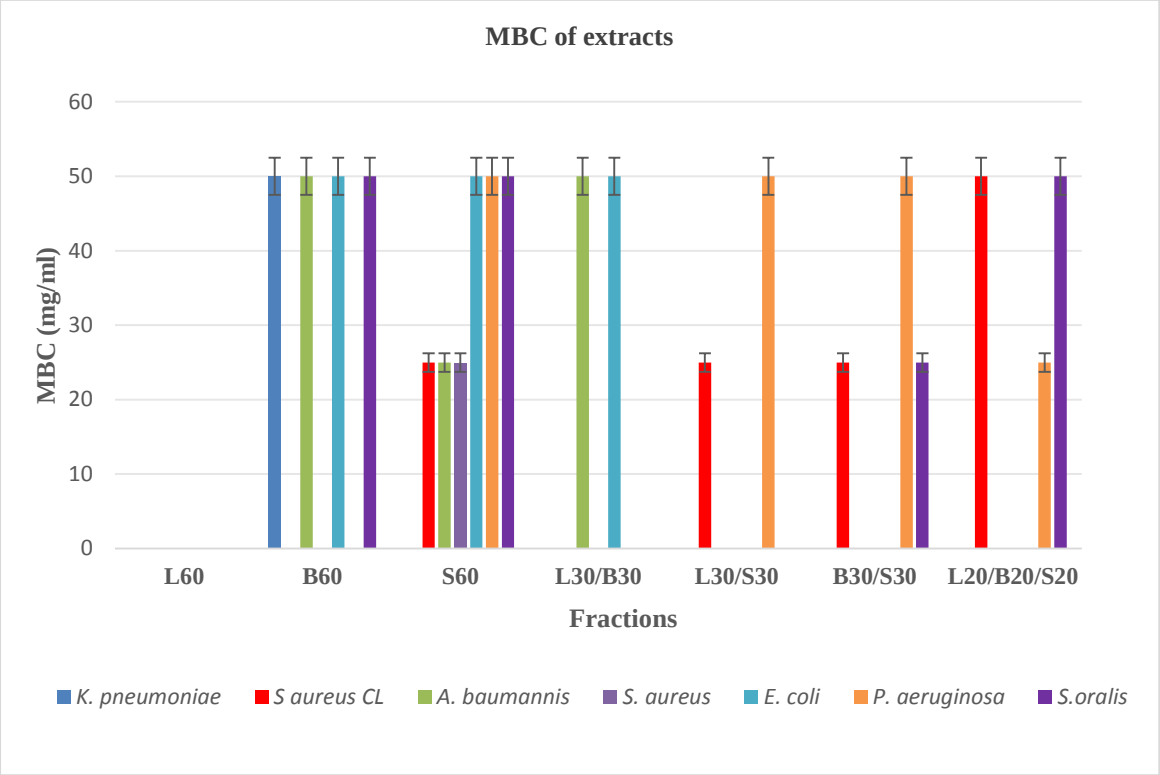

Figure 4. Minimum Bactericidal Concentration (MBC) (mg/ml) of extracts

Among the starting substances, the seed extracts is the most active. It killed six out of seven strains with MBC values between 25 and 50 mg/mL. The next bactericidal extract is that of the steam barks which killed four out of seven strains. It should be noted that gram-negative bacteria were more sensitive to the steam bark extracts. Gram-positive and negative bacteria strains were sensitive to the seed extracts. Seeds have a broad spectrum of action than the barks. Among the mixtures, the B30/S30 extract is the most active, it had a bactericidal effect on three strains with an average value of around 37.5 mg/ml. This is the extract with the lowest average value. The L20/B20/S20 mixture also had an effect on three strains but with a higher average MBC value of 41.66 mg/ml (Figure 5). Both gram-positive and gram-negative strains of bacteria were susceptible to both mixtures. They killed two gram-positives versus one gram-negative.

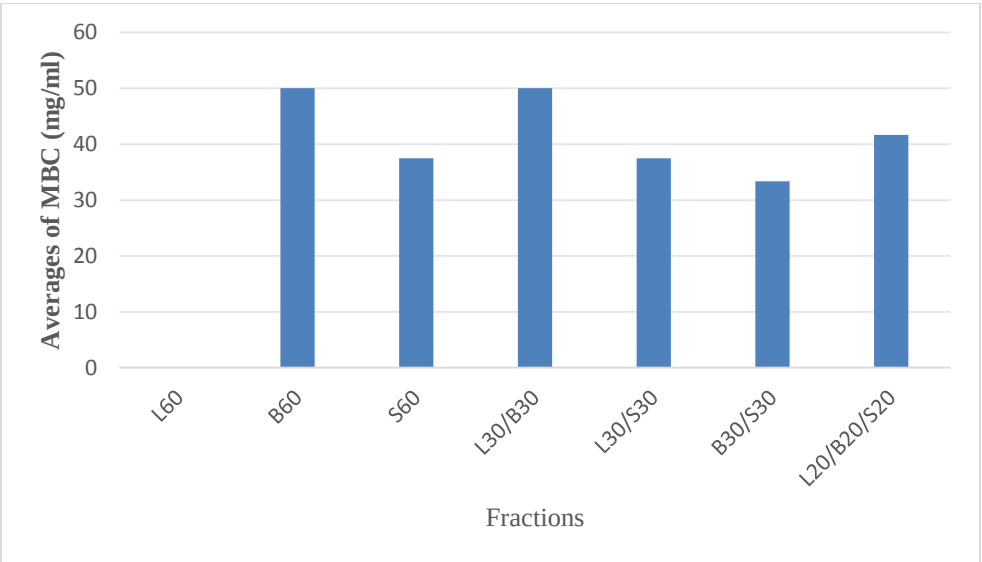

Figure 5. Averages of Minimum Bactericidal Concentration (MBC) (mg/ml) of extracts

The seed extracts kills twice as much as the most active mixture (B30/S30). For the bactericidal effect, only an antagonistic effect is observed. In summary, the seed extract is more effective when it comes to the evaluation of the bactericidal effect.To finalize this work, a phytochemical study was undertaken on the most active fraction (S60 and B30/S30) leading to a qualitative and quantitative identification of the major chemical families of molecules.

Qualitative and quantitative composition of the extracts

The phytochemical screening of the most active fractions (S60 and B30/S30) indicates the presence of Reducing compound, Alkaloids, Flavonoids, Catechy tannins, Gallic tannins, Saponin, Terpenoids, Mucilages, O-heterosides (Table 2). Anthocyanins, Leuco-anthocyanins and Coumarin are present in the mixture and not in the seed extract (Table 2). These last families of compounds would be responsible for the good inhibitory activity noted at the mixture level. They would also be located in the stem barks and absent in the seeds. This would also explain the good inhibitory and bactericidal activity also noted at the stem bark level. But these families would have activity on gram negative bacteria. The quantitative phytochemical study was also conducted on the ethanolic extract of the B30/S30 mixture and S60 previously more active on the strains of microbes. Reducing compound (7.40 ± 0.13), Flavonoids (31.26 ± 0.2) and Gallic tannins (19.70 ± 0.34) are higher at the mixture level than at the seed level. The high level of flavonoids remains a considerable asset for this extract since authors have indicated in the literature that flavonoids are sometimes very interesting antimicrobials [39]. Some constituents are higher in the seeds than in the mixture. These are Alkaloids (9.66 ± 0.22), Saponin (1.98 ± 0.45), and Terpenoids (1.56 ± 0.52). The high level of alkaloids in the seed extract would be responsible for the good bactericidal activity of this organ because it has been shown that alkaloids have very interesting antimicrobial activities [40]. The mixture of the different organs of the mango tree led to the obtaining of fractions with intermediate qualitative and quantitative chemical composition. This mixture led to a significant modification of the quantity of certain large families present in the starting substances. This modification is the basis of the biological activity noted in combinations. It would also be the basis of the appearance of synergistic and antagonistic effects.

Table 1. Phytochemical constituents of more active fractions (B30/S30 and S60) (mg/100g)

Compounds	**QL(B30/S30)**	**QN(B30/S30) (**mg/100g**)**	**QLS60**	**QNS60 (**mg/100g**)**
Reducing compound	+	7.40 ± 0.13	+	0.84 ± 0.02
Alkaloids	+	6.14 ± 0.01	+	9.66 ± 0.22
Flavonoids	+	31.26 ± 0.2	+	6.86 ± 0.20
Tanins catechic	+		+	3.22 ± 0.035
Tanins gallic	+	19.70 ± 0.34	+	
Anthocyanins	+	nd	-	
Leuco-anthocyanins	+	nd	-	**nd**
Quinonics compound	-		-	
Saponin	+	1.54 ±0. 28	+	**1.98** ± 0.45
Coumarin	+	**nd**	-	
Terpenoids	+	1.46 ± 0.64	+	**1.56** ± 0.52
Mucilages	+	**nd**	+	**nd**
Cartenoids	-		-	
Free Anthracenics	-		-	
O-heterosides	+	**nd**	+	**nd**

(+) = Presence; (-) = Absence; **nd**: not determined; **QL**: Qualitative analysis of the extract; **QN**: Quantitative analysis of the extract

IV. CONCLUSION

Mangifera indica is a plant whose organs are highly sought after in the treatment of respiratory infections. The antimicrobial study was conducted on these three organs (leaves, steam bark, seeds). In terms of antimicrobial activity of the organs, the seeds are the first, then the steam bark and finally the leaves. Among the mixtures made, the mixture (seeds/steam barks) is the most active and indicates the appearance of a synergistic effect existing between these organs. This work brings a breakthrough in the search for natural remedies and the recovery of waste in a context where mango seeds constitute environmental pollutants in our country.

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DISCLOSURE OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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