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Influence Of Heat Treatment On The Extraction, Phytochemical Composition And Antimicrobial Activity Of Syzygium Aromaticum Extracts Alone And In Combination

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Abstract:
Background: *Increasing bacterial resistance to conventional antibiotics is driving the exploration of new therapeutic alternatives, particularly those based on natural substances. This study aimed to evaluate the influence of heat treatment on the extraction yields, phytochemical composition, and antimicrobial activity of hydroethanolic extracts of Syzygium aromaticum, alone or in combination with Allium sativum and Garcinia cola.*
Material and Methods: *Two extraction methods were compared: maceration at room temperature (29.4°C) and infusion with the hydroethanolic mixture heated to 72.6°C. Extracts were screened and then tested on seven bacterial strains by determining the inhibition diameters, MIC, and MBC.*
Results: *The results show that heat treatment slightly improves yields, increasing from 11.25% to 11.78% for Syzygium aromaticum. The mixture of Syzygium aromaticum with Allium sativum and Garcinia cola also shows an interesting increase in yield (from 8.75% to 10.02%). Phytochemical analyses reveal a notable increase in several compounds at high temperature, notably flavonoids (from 6.33% to 7.38% for Syzygium aromaticum, and up to 11.72% for the infused mixture). The antimicrobial activity, evaluated on seven strains of microbes by determining the inhibition diameters, improved significantly after infusion, reaching for example 23mm against E. coli and 20mm against P. aeruginosa. The MIC and MBC confirm this trend, with lower values for the infused extracts, in particular the infused mixture, effective against 5 out of 7 strains tested.*
Conclusion: *This heat-enhanced synergy suggests that heat treatment, combined with the use of co-agents, could represent a promising avenue for developing effective natural antimicrobial alternatives.*
Key Word: *Heat treatment; Phytochemical composition; Hydroethanolic extracts; Syzygium aromaticum; Antimicrobial activity.*

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I. Introduction

In Africa, respiratory diseases represent a major public health concern [1,2]. Environmental, climatic, and socioeconomic factors contribute to their prevalence, including pollution, smoke from domestic biomass, and frequent infections [3,4]. Despite medical advances, combating these diseases remains complex due to a lack of resources and limited access to care.

Indeed, access to pharmaceutical drugs to treat these diseases is limited by several obstacles. On the one hand, their high cost makes them inaccessible to a large portion of the population, particularly in rural areas [5,6]. On the other hand, the availability of specific treatments is often insufficient due to poor healthcare infrastructure and an irregular drug supply. Furthermore, the side effects of some treatments, as well as antibiotic resistance observed in certain respiratory infections, pose additional challenges [7,8].

Given these limitations, interest in the use of medicinal plant extracts has grown. Africa has a rich traditional pharmacopoeia, used for centuries to treat various respiratory disorders [9-11]. These substances include cloves, garlic, and cola. These substances are known to be effective against strains responsible for respiratory diseases [12-14]. A synergistic effect between these substances has also been demonstrated in our previous work [15-

[17]. In addition to being more accessible and less expensive, these natural alternatives often offer fewer side effects, although scientific and regulatory oversight is necessary to ensure their efficacy and safety.

Furthermore, temperature has been shown to play a crucial role in optimizing the extraction of active ingredients from a bioactive substance [18-21]. Appropriate temperature variation can impact the chemical composition of extracts, thus influencing their therapeutic potential. The present work aims to study the influence of temperature on the phytochemical composition and antimicrobial activity of *Syzygium aromaticum* and its derivative mixture previously active on microbes identified as being responsible for certain respiratory infections.

II. Material And Methods

Powder Preparation:

The plant material used in this study was purchased at the market in the study area. *Allium sativum* and *Garcinia kola* were peeled. Both plants were thoroughly washed, cut, and then left to dry in an oven at 35°C for 7 days. *Syzygium aromaticum* was also cleaned and dried. The dried substances were carefully ground using an electric grinder. The ground material was sieved and then packaged in glass jars away from moisture for further processing [15]. The hydroethanolic mixture (v/v) served as the solvent for preparing the extracts.

The two extracts considered in this study were those of *Syzygium aromaticum* alone and a combination of *Allium sativum*, *Syzygium aromaticum*, and *Garcinia kola* in the respective proportions of (10; 40; 10). These two fractions were identified in our previous work as the most effective fractions out of a total of seven during maceration [15].

Heat treatment:

During the preparation of the infusion, distilled water was brought to a boil and then mixed (v/v) with 96% ethanol at room temperature. The temperature of the mixture recorded on the thermometer was 72.68 ± 1.40 °C. 600 mL of the solvent obtained was then added to 60 g of the different powders for the preparation of the infusion. The temperature recorded after stirring was 52.60 ± 1.33 °C. The temperature recorded in the absence of heating during maceration for the other mixtures was 29.4 ± 0.56 °C.

The extracts preparation:

60 g of powder of *Syzygium aromaticum* and the combination were crushed and recovered in 600 ml of hydroethanolic mixture (v/v) for 72 hours (in one case at room temperature and in the other at elevated temperature). 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 [22,23].

Preliminary phytochemical screening (Qualitative and quantitative phytochemical screening):

The qualitative phytochemical screening was performed based on coloring or precipitation reactions. It is made directly on the hydro-ethanolic extracts of *Syzygium aromaticum* and the mixture fractions according to Houghton and Raman method (1998) and Harbon (1984) methods [24, 25]. Quantitative phytochemical tests were carried out according to the method used in our previously works and used by other authors [26-29].

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) [30]. Briefly, 1 ml of pre-culture of 18-24 h (106 CFU/ml) enabled planting a box of Petri dishes containing Mueller Hinton agar by flood. After seeding, the sterile Whatman paper discs (5 mm in diameter) were deposited with sterile pliers. 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 [31]. 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 [32]. Briefly, nine concentrations (8000, 4000, 2000, 1000, 500, 250, 125, 62.5 and 31.25 µg/ml) were performed in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (106

CFU/ml). After 24 h of incubation turbidity tubes was examined relative to the control tube containing distilled water and the inoculum (106 CFU/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 [33].

Data processing and analysis:

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

III. Results

Heat Treatment:

Table no 1 highlights the extraction yields of different extracts as a function of extraction temperature. Extraction yields range from 8.75% to 11.78%. *Syzygium aromaticum* alone exhibits a higher extraction yield than the mixture at both temperatures (Table no 1). All extracts have a pasty texture.

Table no 1: Extraction yields, codes, and physical appearance of the extracts

Parameters / Extracts	Extract Codes	Extraction temperature	physical appearance	Extraction yields (%)
Syzygium aromaticum at 29.4 °C	**SAM 29**	29.41 ± 0.56 °C	Past	11.25
Syzygium aromaticum (Infusion) at 52.6 °C	**SAI 52**	52.60 ± 1.33 °C	Past	11.78
Mixture *Alium sativum* (10g), *Syzygium aromaticum* (40g), *Garcinia cola* (10g) at 29.4 °C	**MM 29**	29.41 ± 0.56 °C	Past	8.75
Mixture *Alium sativum* (10g), *Syzygium aromaticum* (Infusion) (40g), *Garcinia cola* (10g) at 52.6 °C	**MI 52**	52.60 ± 1.33 °C	Past	10.02

Qualitative and quantitative composition of the extracts:

Table no 2 presents the phytochemical constituents detected in the extracts of Syzygium aromaticum (SAM 29; SAM 52) alone and of the mixture (MI 29; MI 52) containing Allium sativum, Syzygium aromaticum, and Garcinia cola, at two different temperatures (29.4 °C and 52.6 °C). The percentage of reducing compounds varies from 4.82% for SAM 29 to 6.28% for MI 52. That of alkaloids increases from 2.28% (SAM 29) to 4.84% (MI 52). Flavonoids present percentages ranging from 6.33% (SAM 29) to 11.72% (MI 52). Catechin tannins increase from 6.48% to 10.11%. Saponins vary between 2.55% and 3.52%, and terpenoids between 1.12% and 3.22%. In all cases, an increase in percentages is noted after raising the temperature.

Table no 2: Phytochemical constituents of extracts at different temperatures (%)

Extracts / Compounds	SAM 29		SAI 52		MM 29		MI 52	
	QLA	QNA (%)	QLA	QNA (%)	QLA	QNA (%)	QLA	QNA (%)
Reducing compound	+	4.82 ± 0.04	+	4.99 ± 0.23	+	5.40 ± 0.04	+	6.28 ± 0.23
Alkaloids	+	2.28 ± 0.03	+	2.75 ± 0.03	+	2.14 ± 0.01	+	4.84 ± 0.08
Flavonoids	+	6.33 ± 0.05	+	7.38 ± 0.08	+	11.36 ± 0.12	+	11.72 ± 0.16
Tanins catechic	+	6.48 ± 0.12	+	8.31 ± 0.25	+	9.70 ± 0.23	+	10.11 ± 0.045
Tanins gallic	+		+		+		+	
Anthocyanins	-		-		-		-	
Leuco-anthocyanins	+	nd	+	nd	+	nd	+	nd
Quinonics compound	-		-		-		-	
Saponin	+	2.55 ± 0.44	+	3.33 ± 0.32	+	3.52 ± 0.28	+	3.48 ± 0.32
Coumarin	+	**nd**	+	**nd**	+	**nd**	+	**nd**
Terpenoids	+	1.12 ± 0.06		3.22 ± 0.09	+	0.98 ± 0.06		2.28 ± 0.12
Mucilages	+	**nd**		**nd**	+	**nd**		**nd**
Cartenoids	-		-		-		-	
Free Anthracenics	-		-		-		-	
O-heterosides	+	**nd**		**nd**	+	**nd**		**nd**

(+) = Presence; (-) = Absence; **nd**: not determined; **QLA**: Qualitative analysis of the extract; **QNA**: Quantitative analysis of the extract.

Antimicrobial Activity:

Susceptibility of Standard Antimicrobials Used:

Table no 3 presents the susceptibility profiles of various bacterial strains to ceftriaxone (CTR) and gentamicin (CN). In general, *K. pneumonia, S. aureus, and P. aeruginosa* were susceptible to the standards used. However, *S. aureus* CL, *E. coli, and S. oralis* showed resistance to these standards.

Table no 3: Susceptibility of Various Bacterial Strains to Ceftriaxone (CTR) and Gentamicin (CN)

Bactéries / Standards	***K. pneumoniae***	***S aureus CL***	***A. baumannis***	***S. aureus***	***E. coli***	***P. aeruginosa***	***S.oralis***
Ceftriaxone (CTR)	Sensitive	Resistant	Sensitive	Sensitive	Resistant	Sensitive	Resistant
Gentamycine (CN)	Sensitive	Resistant	Resistant	Sensitive	Resistant	Sensitive	Resistant

Extracts inhibitory diameter zone with the reference strains:

The inhibition diameters obtained for the tested extracts (SAM 29, SAI 52, MM 29, MI 52) on seven bacterial strains reveal varying levels of activity (Figure no 1). Moving from extracts from macerations to extracts from infusions, we observe a sharp increase in diameters, sometimes ranging from no inhibition to the appearance of strong activity. This is precisely the case for bacteria such as: *S aureus* CL (0mm to 18mm), *E. coli* (0mm to 23mm), *P. aeruginosa* (0mm to 20mm), *S. oralis* (0mm to 13mm) in the case of *sygizium aromaticum* (SAI 52) and *A. baumannis* (0mm to 19mm), *E. coli* (0mm to 14mm), *S. oralis* (0mm to 17mm) in the case of the mixture (MI 52).

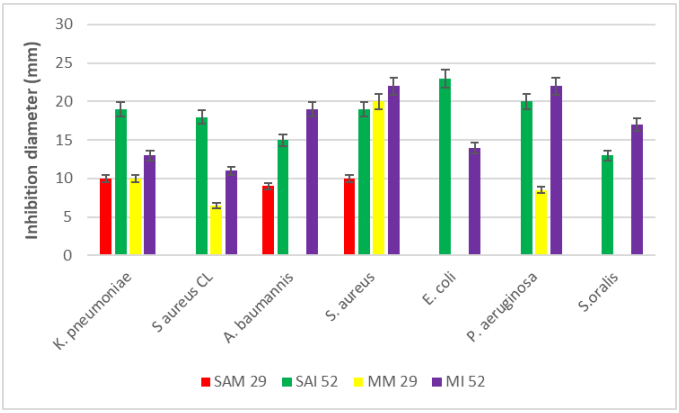

Figure no 1. Diameters of inhibition of different fractions

Minimum Inhibitory Concentrations (MIC) of Extracts:

Figure no 2 shows MIC values (mg/mL) as a function of temperature and formulation (pure clove extract and the clove, garlic, and cola mixture). MIC values range from 2.5 mg/mL to 10 mg/mL. For *Klebsiella pneumoniae*, the MICs are 10 for SAM 29, 7.5 for SAI 52, 10 for MM 29, and 3.75 for MI 52. Against *Staphylococcus aureus* CL, the MICs are 5 for SAI 52 and MI 52, with no values for SAM 29 and MM 29. For *Acinetobacter baumannis*, the MICs are 10 for SAM 29 and 7.5 for SAI 52, with 6.25 for MI 52. Other strains show similar trends.

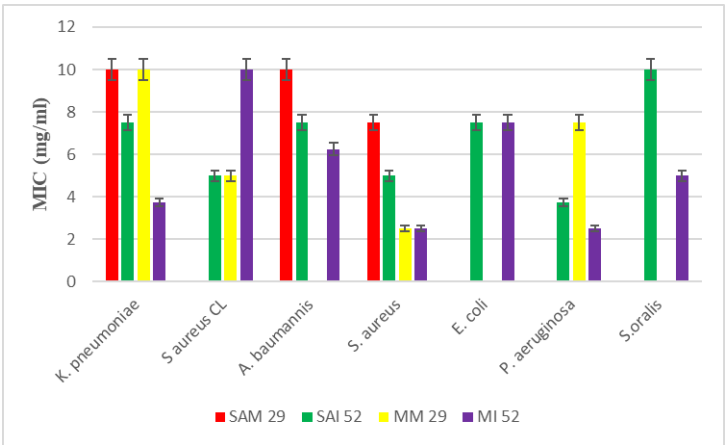

Figure no 2: Minimum inhibitory concentrations (mg/ml) of the extracts

Minimum Bactericidal Concentration (MBC) (mg/mL) of extracts:

Figure no 3 shows the MBCs (Minimum Bactericidal Concentrations) for the various clove extracts, alone or in a mixture, at different temperatures. For *Klebsiella pneumoniae*, the MBC is 50 for SAM 29 and SAI 52, and 10 for MI 52. For *Staphylococcus aureus* CL, the MBC is 25 for SAI 52 and MI 52. For *Acinetobacter baumannis*, the MBC ranges from 25 (SAM 29) to 20 (MI 52). For S. aureus, it is 50 for SAM 29 and SAI 52, and 10 for MM 29. Other strains show similar trends.

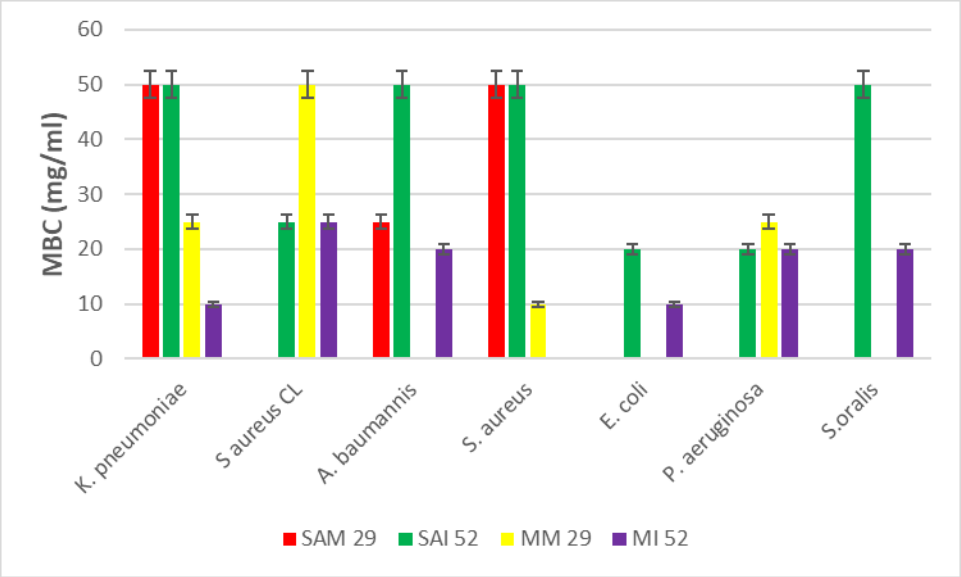

Figure no 4: Minimum Bactericidal Concentration (MBC) (mg/ml) of extracts

IV. Discussion

This study explores the effect of heat treatment on the efficacy of clove (*Syzygium aromaticum*) extracts, alone or combined with garlic (*Allium sativum*) and cola (*Garcinia kola*), against several pathogenic bacteria. The extraction process includes an infusion at approximately 52.60°C from a hydro-ethanolic mixture at a temperature of 72.60°C. This process significantly improves yields compared to maceration at room temperature (Table no 1). Clove alone offers a higher extraction yield than the mixture, likely due to the low contribution of *Allium sativum*. Indeed, in our previous work, *Allium sativum* indicated a very low extraction yield [15]. The addition of this substance to clove would have significantly influenced the extraction capacity by altering the solubility of the compounds or by creating interactions between the different starting constituents through chelation. Indeed, higher temperature could increase the solubility of target compounds and enhance their diffusion into the solvent, which generally results in an increase in extraction yield [34].

Phytochemical analysis revealed that all extracts contained bioactive compounds such as flavonoids, tannins and saponins, with higher concentrations in the extracts obtained after infusion. Both mixtures (MM 29 and MI 52) generally presented higher concentrations for some compounds compared to the *Syzygium aromaticum* extract alone (SAM 29 and SAI 52) (Table no 2). This trend can be attributed to a synergy between the starting substances, as highlighted by Lemoine and Dupont (2025), who observed that certain plant combinations improve the extraction and stability of flavonoids [35]. Flavonoids and tannins levels were the highest. The average level was recorded in the Reducing compounds. The low levels were noted in Alkaloids, Saponin and Terpenoids. The alkaloid level almost doubled when moving from maceration to infusion at the mixture level. Heat would have further solubilized the alkaloid compounds contained in *G. kola* and *A. sativum*. These results show that the solvent temperature of 72.6 °C strongly contributed to the optimization of the extraction conditions, increasing the rate of the different constituents both in the *Syzygium aromaticum* extract and in the mixture. These results are consistent with the studies of Morel and Petit (2024), who demonstrated that higher temperatures promote the extraction of secondary metabolites by increasing their solubility and facilitating their diffusion into the solvent [36]. This could have important implications for their pharmacological applications.

Antimicrobial activity was assessed by measuring inhibition diameters and determining MICs and MBCs. Ceftriaxone (CTR) and gentamicin (CN) were previously used as standard antibiotics to assess the susceptibility of the microbial strains used. These antibiotics are well-known standards with established antimicrobial activity profiles. Gentamicin (an aminoglycoside) is effective against Gram-negative bacteria, while Ceftriaxone (a third-generation cephalosporin) has a broad spectrum of activity, covering both Gram-positive and Gram-negative bacteria [37]. The effects of the two standards converge towards the same result, except in the case

of *A. baumannis* which is the only strain sensitive to Ceftriaxone (CTR) and resistant to Gentamycin (CN) (Table no 3). During the tests with the extracts made, strains such as *S. aureus* CL, *E. coli* and *P. aeruginosa*, initially resistant, become sensitive to the extract obtained after infusion (Figure no 1), highlighting the key role of heat in the release of the active compounds identified in the phytochemical quantification (Table no 2). The temperature used during the infusion is approximately 52 °C, ideal for the extraction of certain families of compounds such as polyphenols, tannins and flavonoids and terpenoids. The quantitative growth of phytochemical compounds therefore led to an increase in antimicrobial activity (Figure no 1). These values also corroborate those of Nguyen et al. (2023), who reported diameters of 18–25 mm for similar polyphenolic extracts against *S. aureus* and *E. coli* [38].

The determination of MICs confirms that heating the pure clove extract modifies the activity in a contrasting way. Heating the mixture largely optimizes the antibacterial effect, in particular against Gram negatives, while maintaining good activity on most Gram positives. The mixture has a broad spectrum of action and therefore remains the most promising formulation, with the lowest MICs for six strains out of seven strains tested. Only *S. aureus* CL loses effectiveness in the heated mixture. Regarding the bactericidal effect, the mixture obtained after infusion kills six out of seven germs with the lowest values observed during this work. The one obtained after maceration at room temperature kills four out of seven germs with relatively higher values. The clove extract alone at high temperature had an effect on the seven germs with higher values. At room temperature, this extract had an effect on only three germs. These observations support the results obtained above during the determination of the inhibition diameter and the MIC. Overall, the extracts obtained after infusion (SAI 52 and MI 52) present higher concentrations of bioactive compounds such as flavonoids, catechin tannins, alkaloids, reducing compounds, and terpenoids. This richness in secondary metabolites is correlated with better antibacterial efficacy, as evidenced by the lower MIC and MBC values, as well as larger inhibition diameters.

V. Conclusion

Heat treatment significantly improves the extraction yields, phytochemical composition, and antimicrobial activity of the extracts studied. Infusions, carried out at approximately 52°C, promote the extraction of bioactive compounds such as flavonoids and tannins, contributing to greater efficacy against various bacterial strains. The combination of substances (cloves, garlic and cola) enhances this action through a synergistic effect.

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