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Assessment of Total Terpenoids and Potential Synergistic Antimicrobial Activities of *Avena sativa* and bark of *Carica papaya*

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ABSTRACT

Background: Triterpenoids derived from isoprene units, with diverse medicinal uses acting as a vital compound in ethnomedicine and modern drug research. The aim of this study is the assessment of potential synergistic activity of n-hexane fractions of A. sativa and methanolic fraction of C. papaya in combination with ketoconazole and ciprofloxacin against test organisms.
Method: About 800 g of Avena sativa (oat) and 500 g of Carica papaya were extracted with 500 mL each of 80% methanol, n-hexane, ethyl acetate and chloroform separately. Qualitative and quantitative evaluation of triterpenoids was determined. The samples extract were fractionated using column chromatography. The antimicrobial susceptibility assessment was done using agar well diffusion method while minimum inhibitory concentration (MIC) was performed using broth microdilution method. Evaluation of synergistic potential of A. sativa and C. papaya bark was done using agar diffusion checker board (ADCB) method.
Results: The result shows the presence of terpenoids in both samples with total terpenoids in A. sativa (9.31%) and C. papaya (2.18%) respectively. Synergistic activity of A. sativa in combination with ketoconazole was observed at 10:0, 1:9 and 0:10 combinations. Combination that shows synergistic activity of bark of C. papaya combined with ketoconazole is 10:0, 1:9 and 0:10 respectively against C. albicans.
Conclusion: A combination of n-hexane fraction of A. sativa and methanolic fraction of bark of C. papaya and Ketoconazole possesses synergistic activity against C. albicans while combination of plant extract and ciprofloxacin showed no synergistic activity against bacterial isolates.
Keywords: Synergistic, antimicrobial, chromatography, susceptibility, Avena sativa, Carica papaya

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INTRODUCTION

Medicinal plants provide a veritable robust and rich source of bioactive constituents with diverse therapeutic benefits and are reported to be safe, efficacious, accessible and economical. Ethnomedicinal uses of medicinal plants in the treatment of Otitis media, diarrhea, ulcers, wounds, tinea capitis, gonorrhea etc indicate their antimicrobial potential.[1]

Globally, there is a damning challenge in history of therapeutics as multidrug resistant (MDR), pathogens continue to soar especially with the use of sub-clinical doses, indiscriminate and inappropriate uses of antimicrobials synthetic drug in the treatment of infection that result in recurrent infections and therapeutic failure[2]. This not only increases the cost of treating microbial infections but mortally and morbidity. The use of natural product becomes more relevant and compelling as cheaper, safer and more accessible alternatives as antimicrobials.[3]

Triterpenoids derived from isoprene chemical rings, with diverse medicinal uses like antimicrobial acting as a vital compound in ethnomedicine and modern drug research. *Carica papaya* is a soft stemmed, herbaceous plant with a hallow cylindrical trunk with large leaves. The fruits are large freshly and oval[4]. *Avena sativa* usually called oat is a functional food with both nutritional and medicinal uses and is reported to have antimicrobial activity[5]. The aim of this study is the determination of total terpenoids and potential synergistic antimicrobial activity of the bark of *Carica papaya* and *Avena sativa.*

MATERIALS AND METHOD

Materials/Reagent: Electric blender, sterile plastic bottles, sterile distilled water, sterile aluminum tray, 80% methanol, n-hexane, chloroform, ethyl acetate, rotary evaporator, soxhlet apparatus, sulfuric acid, petroleum ether, whatman filter paper, electronic balance, ceramic mortal, vanillin, oven, stop clock, autoclave, UV spectrophotometer, tween 20.

Stock Culture of Bacteria/Fungi Strain: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*

Sample collection and preparation: One tin of Quacker Oat™ containing 100% whole oat grain packed by Pepsico Foods Nigeria Limited, Lagos, Nigeria, was purchased from a supermarket in Abraka, Delta State, Nigeria on the 30th of November, 2025. Fresh bark of *Carica papaya* grown in a residential area in Abraka, Delta State, Nigeria was collected using a sharp knife on the 30th of November, 2025 and thoroughly washed under running tap water and rinsed by sterile distilled water. The washed *C. papaya* bark was then placed on a sterilized aluminum tray and air dried at ambient temperature for about a week when the weight became constant. The dried samples were ground to fine powder using an electric grinder and stored in a sterile plastic bottle at 4℃.

Sample Extraction: About 800 g of *Avena sativa* and 500 g of *Carica papaya* were extracted with 500 mL each of 80% methanol, n-hexane, ethyl acetate and chloroform using soxhlet apparatus at a temperature of 45℃ for 8 hours each and concentrated using R110 rotary evaporator at 40℃. The extracts were then subjected to another round of extraction using 500 mL of distilled water at 60℃ for 6 hours and the extract filtered into 500 mL round bottom flask and freeze dried[6].

Evaluation of Total Terpenoids

Qualitative Analysis: The presence of triterpenoids in methanol extract of bark of *C. papaya* and *A. sativa* was done using Salkowski's test. 5 mL of plant extract was dissolved in 2 mL of chloroform in a test tube, concentrated sulfuric acid was carefully added along the side of the test tube and a reddish brown lower layer was observed in both samples indicating the presence of triterpenoids[7].

Quantitative Assessment of Total Terpenoids: About 100 mg of dried methanol extract of each plant extract was weighed using an electronic balance and soaked in 10 mL of ethanol for 24 hours in the dark and filtered using Whatman No 1 filter paper. The filtrate was further extracted with 20 mL of petroleum ether using a separating funnel and shaken vigorously for one minute and allowed to stand until complete separation was observed. The ethanol phase was drained. The ether phase was separated in a pre-weighed glass vials and allowed to dry completely and ether was evaporated on a water bath[8]. The yield of total terpenoid was calculated using the formula;

$$Percentage\ yield = \frac{Wti - Wtf}{Wti} \times 100$$

Where:

Wti = initial weight of the dried plant extract

Wtf = weight of terpene fraction left after complete drying of ether phase

The dried terpenoids extract was then weighed using electronic balance and its value expressed in mg/g. This

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procedure was repeated in triplicate and the mean value taken

***Fractionation and Purification of the Extracts*:** The n-hexane, chloroform, methanol and ethyl acetate extracts were subjected to column chromatography to fractionate the extract using a column size 3.5 x 19 cm. 80 g of silica gel was used as stationary phase and varying solvents combination of gradual increasing polarity (n-hexane, methanol, chloroform and ethyl acetate) as mobile phase. The wet packing method was used in packing the glass column. The glass column used for this experiment was held up vertically using a retort stand after stocking the lower column with glass wool using a sterile glass rode. The slurry for the n-hexane extract was prepared by mixing 200 g of silica gel with 400 mL of 80% methanol. Similarly, the slurry for chloroform, methanol and ethyl acetate was prepared using n-hexane, ethyl acetate and chloroform respectively for each of the samples. The slurry was poured down carefully into the column with the tap opened to allow free flow of solvent in the beaker below, ensuring that the silica gel and the glass wool was not mix with the solvent 24 hours after the tap was locked. The solvent at the top of the gel was allowed to flow down to the silica gel meniscus.

About 15 g of the concentrated and dried extract of each sample was thoroughly mixed using a ceramic mortal with 28 g of silica gel until a homogenous powder was formed, loaded and gently layered on top of the column. A layer of 12 g silica gel was added on top the column to prevent direct contact with eluent. Elution of the extract was done using a solvent system of gradually increasing polarity in the following ratio 1L n-hexane: ethyl acetate, 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. The same ratio was used for ethyl acetate: methanol combination for each sample. The eluents were collected in sterile 100 mL bottles and allowed to evaporate to dryness at ambient temperature[9] and further purified using analytical thin layer chromatography.

***Analytical thin layer purification and bulking of fractions*:** Preparative F254 silica gel (1 cm) precoated on aluminum plate was used as stationary phase and n-hexane: ethyl acetate (7:3) as mobile phase for thin layer analysis of fractions of both samples. The thin layer plate was then developed in a glass chromatographic tank containing the solvent systems. A glass lid was used to cover the tank and the solvent allowed to ascend until about 25% the length of the plate. The TLC plate was then removed and dried under hot air oven, viewed under UV visible lamp at 365 and 254 nm to identify the fluorescing spot, marked and sprayed with 0.16 g vanillin in 40 mL concentrated sulfuric acid. The plate was placed in a hot air oven at 100℃ for 4 hours using a stop clock and the color reaction recorded, the retention factor (Rf) value was determined using the formula described by Sherma and Fried.[10]

$$Rf = \frac{\text{Distance travelled by the sample from the starting point}}{\text{Distance travelled by the solvent from the starting point}}$$

From the Rf value, fractions with similar TLC mobility were bulked into a preweighed 100 mL beaker. The bulked fractions were further analyzed to access the number of spots and concentrated at 30℃ for *A. sativa* fractions and 40℃ for *C. papaya* fractions and weighed using an electronic weighing balance (Drawell TD-A electronic weighing balance).[9]

Preparation of Growth Media

Nutrient Agar: About 14 g of Himedia nutrient agar was weighed using electronic balance and dissolved in 500 mL of distilled water in a 1L round bottom flask, stirring well using a glass rod and heated gently until a clear solution was obtained and pH adjusted to about 7.0 and seal with screw cap. The solution was then autoclaved at 121℃ for 15 minutes for complete sterilization. The agar was then cooled to solidify at room temperature and stored at 4℃ in a refrigerator.

Preparation of Sabourand Dextrose Agar (SDA): About 20 g of dextrose, 5 g of peptone and 7.5 g of agar was weighed using electronic weighing balance and suspended in 400 mL distilled water in a round bottom flask. The mixture was heated using a hot plate with frequent stirring to boiling until a clear solution was formed. The pH was adjusted to 6.0 with few drops of dilute sodium hydroxide. The medium was then autoclaved for 15 minutes at 121℃ and the sterile medium allowed to cool to 45℃. The mixture was then poured into a sterile petri dish and the plate allowed to solidify for 40 minutes and stored in a refrigerator at 4℃.

Standardization of Bacteria and Fungi Inoculums: Each strain of *E. coli*, *S. aureus*, *P. aeruginosa* were sub cultured for 12 hours at 34℃ in nutrient agar slants. The growth was then harvested using 6 mL of sterile saline water. The absorbance was adjusted at 580nm and diluted using UV-visible spectrophotometer.

The fungi inoculum was prepared by growing the *C. albicans* using SDA plate and allowed to fully mature for 4 days at 37℃. The sterile distilled water was mixed with 0.1% tween 20 to disperse the spores, and the liquid

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added to fungi plate. The spores were gently scraped using a sterile spatula and transferred to autoclaved tube. The suspension was stirred thoroughly and filtered using Whatman 1.0 filter paper. A UV spectrophotometer was then used to count spores by measuring its turbidity and concentration adjusted to 1.2 x 10^6 spore cell/mL.

In-vitro Antimicrobial Susceptibility Assessment of Pure Fractions: The antimicrobial assessment of the pure plant fractions was performed according to the method of Clinical Laboratory Standard Institute[11] using the agar well diffusion method. A sterile cotton swab was used to streak aseptically the standardized bacteria culture over the whole surface of a nutrient agar to create a uniform lawn of growth. A sterile cock borer was then used to bore a 6 mm diameter well on the solidify agar. 100 µL of 200 mg/mL of the purified plant fraction using ciprofloxacin as positive control and sterile distilled water as negative control to different wells. The plant extract was allowed to diffuse into the agar for 30 minutes and the plate incubated in 50L Gentab incubator in an upright position for 24 hours at 37℃. The zone of inhibition was measured in millimeter from one edge of the zone to the opposite edge using a caliper. Clear zone of less than 12 mm is regarded as resistant, 13 – 17 mm moderate sensitivity and 18 – 20 mm sensitive. This procedure was repeated using SDA for antifungal assessment using Ketoconazole and distilled water as positive and negative control respectively.

Determination of Minimum Inhibitory Concentration of fractions: Evaluation of minimum inhibitory concentration (MIC) of purified fractions was performed using broth microdilution methods. Two-fold serial dilution of sample purified fractions in sterilized nutrient broth were prepared in concentrations of 100, 50, 25, 12.5 and 6.25 µg/mL. The 100 µg/mL of the concentration was made by adding 4 mL containing 0.4 mg of the fraction and dissolved in 2 mL of polyoxyethylene (20) surbittan monolaurate and 95% ethanol to aid solubility and uniform dispersion on the agar plates, the standardized microbial inoculum was then added and incubated using ciprofloxacin as positive control for bacteria, Ketoconazole for fungi and pure plant extract as negative control. The lowest concentration showing no visible growth was recorded.

Assessment of Synergistic Potential of Pure Fractions with Standard Antimicrobials: Evaluation of synergistic potential of *A. sativa* and *C. papaya* bark was done using agar diffusion check board (ADCA) method as described by the Clinical and Laboratory Standard Institute[11] by assessing the effect of the fraction in combination with ciprofloxacin and ketoconazole against the bacteria and fungi respectively the overlay inoculum susceptibility disc method. Different concentration of the fraction and ciprofloxacin, were prepared using the continuous variation checker board method. The fractional inhibitory concentration (FIC) is assessed by dividing the MIC of each of the fractions in combination by the MIC of fraction alone and summation of the FIC gives the FIC index, which was used to classify the effect of the combination ratio as addictive, synergistic, antagonistic or indifferent concentration of the extract and ciprofloxacin disc were prepared in the ratio of 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0. This 2 fold serial dilution was inoculated with 0.1 mL of standard test organisms, and the plates kept for one hour at room temperature to allow for pre-diffusion and then incubated for 24 hours at 37℃. The synergistic potential was assessed algebraically by evaluating the FIC indices according to the equation below;

$$\text{FIC index} = \text{FIC fraction} + \text{FIC ciprofloxacin} \ldots \text{eqn 1}$$

$$\text{FIC fraction} = \frac{\text{MIC of fraction in combination with ciprofloxacin}}{\text{MIC of fraction alone}} \ldots \text{eqn 2}$$

$$\text{FIC ciprofloxacin} = \frac{\text{MIC of combination with ciprofloxacin with fraction}}{\text{MIC of ciprofloxacin alone}} \ldots \text{eqn 3}$$

The same equation was applied for ketoconazole

RESULTS

The result of the evaluation of total terpenoids and assessment of potential synergistic antimicrobial activities of the bark of *Carica papaya* and *Avena sativa* are presented in the tables below.

Table 1: Qualitative Analysis of Triterpenoids

Samples	Triterpenoids
Avena sativa	+
Carica papaya	+

Key: + = present, - = absent

Table 2: Quantitative Evaluation of Total Terpenoids (%)

Samples	Triterpenoids
Avena sativa	9.31
Carica papaya	2.18

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Table 3: *In-vitro* Antimicrobial Susceptibility Assessment of Pure Fractions

Plant sample	Solvent	Zones of inhibition			
		E. coli	***S. aureus***	***P. aeruginosa***	***C. albicans***
A. sativa	Methanol	1.20±0.31	2.30±0.05	0.90±0.20	12.60±0.03
	n-hexane	2.30±0.01	5.23±0.01	1.20±0.03	13.50±0.05
	Ethyl acetate	0.90±0.01	2.10±0.02	0.80±0.04	11.50±0.10
	Chloroform	1.10±0.03	2.50±0.02	0.90±0.01	12.00±0.03
C. papaya	Methanol	12.40±0.03	12.80±0.04	9.50±0.05	10.50±0.06
	n-hexane	10.50±0.03	12.30±0.13	5.50±0.20	12.30±0.03
	Ethyl acetate	11.80±0.03	13.30±0.02	7.40±0.40	12.10±0.05
	Chloroform	8.30±0.05	12.20±0.06	5.30±0.30	11.80±0.03
Ciprofloxacin		26.40±0.01	27.50±0.03	26.00±.003	-
Ketoconazole		-	-	-	28.50±0.01
Distilled water		0.0	0.0	0.0	0.0

Data is presented in Mean±SD, n=3

Table 4: Assessment of Minimum Inhibitory Concentration of Fractions of *A. sativa* and *C. papaya*

Plant sample	Fractions	Concentration (µg/mL)	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*
A. sativa	Methanol	100	2.5	6.5	1.5	13.0
		50	2.0	5.8	1.2	12.5
		25	1.5	4.5	0.9	10.6
		12.5	1.0	3.0	0.6	6.3
		6.25	0.7	2.0	0.4	4.5
	n-hexane	100	3.5	6.3	1.5	14.5
		50	3.0	5.8	1.1	12.3
		25	2.3	4.9	0.8	10.5
		12.5	1.5	3.6	0.6	8.4
		6.25	0.9	2.5	0.4	6.5
	Ethyl acetate	100	1.5	3.2	1.2	12.1
		50	1.3	2.6	0.9	11.5
		25	0.9	1.8	0.7	10.1
		12.5	0.6	1.5	0.4	8.3
		6.25	0.4	1.0	0.3	6.5
	Chloroform	100	1.8	3.6	1.3	13.1
		50	1.2	3.1	1.0	11.8
		25	0.9	2.5	0.8	10.5
		12.5	0.6	2.0	0.5	8.3
		6.25	0.4	1.5	0.3	5.5
C. papaya	Methanol	100	13.3	13.5	1-.3	11.6
		50	12.1	12.3	9.5	10.4
		25	10.8	10.5	7.8	8.5
		12.5	8.3	8.3	5.9	6.9
		6.25	6.2	6.3	3.0	5.3
	n-hexane	100	11.8	13.4	6.7	13.2
		50	10.3	12.3	5.8	12.3
		25	8.8	10.1	4.5	10.5
		12.5	6.5	8.2	3.1	8.6
		6.25	4.2	5.4	1.8	7.5
	Ethyl acetate	100	10.5	14.1	9.5	13.6
		50	8.7	12.9	7.5	12.5

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Plant sample	Fractions	Concentration (µg/mL)	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*
		25	5.3	10.8	5.6	10.4
		12.5	3.8	8.5	3.5	8.3
		6.25	2.0	6.3	0.2	5.2
	Chloroform	100	10.2	13.8	6.7	11.5
		50	9.3	12.6	5.3	10.4
		25	7.5	10.5	3.1	8.5
		12.5	5.1	7.5	2.0	5.6
		6.25	3.2	4.8	1.6	4.3

Table 5: Synergistic potential of *A. sativa* hexane fractions in combination with ketoconazole against *Candida albicans*

Combination ratio	MIC ketoconazole (mg/mL)	MIC *A. sativa* (mg/mL)	FIC Ketoconazole	FIC *A. sativa*	FIC index	Synergistic potential
10:0	0.155	0.68	-	0.02	0.21	Additive
9:1	0.142	1.56	0.5	0.06	0.98	Additive
8:2	0.130	2.80	0.75	0.15	0.91	Additive
7:3	0.110	4.10	0.68	0.18	0.86	Additive
6:4	0.930	6.20	0.54	0.22	0.76	Additive
5:5	0.156	12.70	0.90	0.58	1.48	Indifference
4:6	0.128	14.50	0.75	0.65	1.40	Indifference
3:7	0.094	17.30	0.57	0.78	1.35	Indifference
2:8	0.060	19.80	0.38	0.86	1.24	Indifference
1:9	0.030	22.30	0.19	0.11	0.30	Synergistic
0:10	0.010	24.50	0.05	0.06	0.11	Synergistic

Table 6: synergistic potential of bark of *C. papaya* methanol fractions in combination with ketoconazole against *Candida albicans*

Combination ratio	MIC ketoconazole (mg/mL)	MIC *A. sativa* (mg/mL)	FIC Ketoconazole	FIC *A. sativa*	FIC index	Synergistic potential
10:0	0.25	0.57	-	0.012	0.17	Synergistic
9:1	0.17	1.23	0.71	0.048	0.73	Additive
8:2	0.95	2.32	0.69	0.10	0.79	Additive
7:3	0.83	3.85	0.55	0.12	0.67	Additive
6:4	0.75	5.30	0.93	0.16	0.59	Additive
5:5	0.08	11.50	0.78	0.43	1.21	Indifference
4:6	0.073	12.30	0.63	0.48	1.11	Indifference
3:7	0.88	15.10	0.42	0.65	1.07	Indifference
2:8	0.053	17.30	0.24	0.71	0.95	Additive
1:9	0.021	18.10	0.09	0.08	0.17	Synergistic
0:10	0.91	22.30	0.02	0.03	0.06	Synergistic

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Table 7: Synergistic potential of ciprofloxacin and n-hexane fraction of *A. sativa* against some bacteria strains

Combination ratio	n-hexane fractions			
	E. coli	***S. aureus***	***P. aeruginosa***	**Synergistic potential**
	FIC index	**FIC index**	**FIC index**	
10:0	-	-	-	Indifference
9:1	1.12	0.95	2.07	Indifference
8:2	4.13	0.90	5.03	Antagonistic
7:3	2.12	3.61	5.73	Antagonistic
6:4	16.31	0.71	17.02	Antagonistic
5:5	2.22	2.83	5.05	Antagonistic
4:6	8.31	1.30	9.61	Antagonistic
3:7	0.61	1.11	1.71	Indifference
2:8	2.45	0.48	2.93	Indifference
1:9	1.35	0.70	2.05	Indifference
0:10	0.90	0.58	1.48	Indifference

Table 8: Synergistic potential of ciprofloxacin and methanol fraction of bark of *C. papaya* against some bacterial stains

Combination ratio	Methanol fractions			
	E. coli	***S. aureus***	***P. aeruginosa***	**Synergistic potential**
	FIC index	**FIC index**	**FIC index**	
10:0	-	-	-	Indifference
9:1	0.91	0.83	1.74	Indifference
8:2	4.00	0.78	4.78	Antagonistic
7:3	1.98	3.23	5.21	Antagonistic
6:4	15.10	0.64	15.74	Antagonistic
5:5	1.95	2.45	4.40	Antagonistic
4:6	7.92	1.12	9.04	Antagonistic
3:7	0.55	0.88	1s.43	Indifference
2:8	2.13	0.31	2.44	Indifference
1:9	1.10	0.65	1.75	Indifference
0:10	0.89	0.49	1.33	Indifference

DISCUSSION

Analysis of Terpenoids: Triterpenoids are a large diverse group of organic compounds found mainly in plant with isoprene units. Triterpenoids are found in both *Avena sativa* and *Carica papaya* extract as shown in Table 1 but with a higher percentage of 9.3% in *A. sativa* than 2.18% in *C. papaya* as shown in Table 2. This may be one of the factors responsible for the antimicrobial activities of both studied samples

Fractionation and purification of extract of A. sativa and C. papaya: Column chromatographic fractionation and TLC purification of extract was performed to separate useful bioactive compounds and exclude phytochemicals that may be devoid of medicinal uses. Fractions of *A. sativa* extract contain mainly lipophilic fractions and *C. papaya* hydrophilic. The lipophilic fractions in *A. sativa* may be due to the presence of esters[12]. Triterpenoids found in both samples may be responsible for their antimicrobial activities.

In-vitro antimicrobial susceptibility assay of pure fractions: The n-hexane and methanol fractions of *A. sativa* and *C. papaya* fractions shows moderate activity against *Candida albicans* with inhibition zone diameter (IZD) of 13.5±0.05 and 12.6±0.03 respectively using the CLSI standard. Apart from methanolic fraction of *A. sativa,* other fractions show no activity against *C. albicans*. Also, methanol and ethyl acetate fractions of *C. papaya* fraction show moderate activity against *E. coli* and *S. aureus* with IZD of 12.4±0.03 and 13.3±0.02 respectively with *A. sativa* fraction showing no activity against the test

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bacteria strains. Both studied samples show no antibacterial activity against *P. aeruginosa*.

Assessment of Minimum Inhibitory Concentration of Fraction of A. sativa and C. papaya: According to the Clinical Laboratory Standard Institute (CLSI, 2017) the minimum inhibitory concentration in determination of antimicrobial activity is taken to be the lowest concentration of a plant extract that totally inhibit the visible growth of microorganism. *In-vitro* from Table 4 visible growth was observed in all test organisms at the highest concentration of 200 mg/mL of extract of both *A. sativa* and *C. papaya*, this implies that the MIC of fractions of both samples may be well above 100 mg/mL. The MIC of ciprofloxacin against common bacteria pathogens is often ≤ 0.5 – 1 μg/mL while the MIC of ketoconazole against *Candida albicans* is about 0.95 μg/mL, therefore, the drug used as positive control has more antimicrobial activities than the fractions of the studied samples.

Evaluation of Synergistic Potential of n-hexane Fractions of A. sativa and Methanolic Fractions of C. papaya against Test Bacteria Isolates and Candida albicans: The synergistic effects of n-hexane fractions of *A. sativa* and methanolic fraction of *C. papaya* in combination with ciprofloxacin and ketoconazole against tested bacteria organisms and *C. albicans* respectively using the FIC index. The synergism was observed in 10:0, 1:9 and 0:10 combinations of fractions of *A. sativa* and ketoconazole against *Candida albicans*. Combination of 9:1, 8:2, 7:3, 6:4 have additive effect while 5:5, 4:6, 3:7 and 2:8 are indifference.

The combination of fraction of *C. papaya* and ketoconazole against *C. albicans* in the ratio of 10:0, 1:9 and 0:10 are synergistic. Additive effects are produced from combination of 9:1, 8:2, 7:3, 6:4 and 2:8, other combination of 5:5, 4:6 and 3:7 are indifference in activity. The results shows that n-hexane fraction of *A. sativa* and methanolic fraction of *C. papaya* have antifungal effect against *C. albicans* and a potentiation effect were observed when combined with ketoconazole. This may enhance the efficacy, reduction in treatment duration and prevention of therapeutic failure in the management of infections caused by *C. albicans*.

The combination of n-hexane fraction of *A. sativa* and ciprofloxacin did not produce any synergistic effect against the studied organism rather the combined activities are either indifferent or antagonistic and therefore must be counterproductive. In Table 8 the combined activity of methanolic fraction of *C. papaya* and ciprofloxacin in the assessment of tested organism is of no clinical benefit as the combination effect are either indifferent or antagonistic. In this antimicrobial combination study the FIC index was determined by adding the FICs of samples and the FICs of the standard drug. FIC index of less than or equal to 0.5 was taken as synergistic, ≤ 1.0 additive, ≤ 4.0 indifference and > 4.0 antagonistic.

CONCLUSION

The dearth in discovery of new allopathic antimicrobial agents has necessitated the investigation of combining plant extract with conventional antimicrobial drugs to explore their possible potentiation of their activities in the treatment of infectious diseases. This study assesses the potential synergistic effect of n-hexane fractions of *A. sativa* and methanolic extract of the bark of *C. papaya* in combination with ciprofloxacin and ketoconazole against some bacteria isolates and *C. albicans* respectively. Both studied sample fractions possess activity against *C. albicans*. Hexadecanoic acid, ethyl ester, 9,12-octadecadienoic acid (Z,Z) methyl ester and 9-octadecanoic acid (Z) nethyl ester identified by Onyeloni *et al.*[12] in *A. sativa* have antifungal activities against *C. albicans*. In this study, a combination of n-hexanne fraction of *A. sativa* and methanolic fraction of bark of *C. papaya* possesses synergistic activity against *C. albicans* in combination with Ketoconazole. The combination of the plant fractions with ciprofloxacin did not show any synergistic activity against *E. coli*, *P. aeruginosa* and *S. aureus*. Further investigation needs to be done on the synergistic activity of fractions of *A. sativa* in combination with conventional antifungal drugs to improve their efficacy, reduce treatment duration and prevent the occurrence of therapeutic failures and recurrent infection.

Declarations

Consent for the publication: The decision to publish was taken by all the authors and the corresponding author was empowered to submit the article of the authors' behalf.

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