



**SYNERGISTIC EFFECT OF *IRVINGIA GABONENSIS* AND *PSIDIUM GUAJAVA*  
LEAVES AGAINST DIARRHOEA CAUSING AGENTS.**

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**ABSTRACT**

Synergistic combinations of antimicrobial agents with different mechanisms of action have been introduced as more successful strategies to combat infections involving diarrhoea. In this study, we investigated synergistic antimicrobial activities of *Irvingia gabonensis* and *Psidium guajava* leaves extracts which are commonly used plants with different antimicrobial agents. Antimicrobial susceptibility of some bacterial and fungi were tested against the combination of different ratios of *I. gabonensis* and *P. guajava* extracts. Minimum inhibitory concentrations (MIC<sup>S</sup>) were determined by agar diffusion method. Plant extracts were tested for synergistic antimicrobial activities with different antimicrobial agents by checkerboard titration. In the results, the disc diffusion method showed synergistic effect between *I. gabonensis* and *P. guajava* against tested organisms. FIC value obtained by checkerboard assay showed less than 1 in combination, this indicating synergistic interaction between the two plants. The study suggest that the combination of the two plants could perform better in the management of diarrhoea than monotherapy.

**KEYWORDS:** Diarrhoea, Synergistic effects, *Irvingia gabonensis*, Checkerboard assay, *Psidium guajava*.

**INTRODUCTION**

Plants have formed the basis of sophisticated traditional medicine and their natural products led for new drug development (Newman et al., 2000). A medicinal plant as described by the World Health Organization (WHO,2005), is a plant which one or more of its parts or organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Medicinal plants have been used for centuries before the advent of orthodox medicine. They have been proved to contain pharmacologically active ingredients which have over the years been exploited in traditional medicine for the treatment of various human and animal diseases (Adamu et al., 2006).

Diarrhoea can be defined as the increased frequency of bowel movements, accompanied by a loose consistency of stools. Diarrhoea results from hyper-peristalsis of the small intestine or colon. Large amounts of Na<sup>+</sup> and K<sup>+</sup> and water are washed out of the colon and small intestine in diarrhoea stools, causing dehydration, hypokalaemia and eventually shock and cardiovascular collapse.

Diarrhoea patients may report frequent loose or watery stool, defaecation usually more than three times per day, often accompanied by pain and abdominal cramping.

In recent years, emphasis on the treatment of diarrhoea has focused on oral rehydration therapy. However, there is still need for a continuing search for effective anti-diarrhoea drugs without side effects and as adjunct to ORT.

Diarrhoea is one of the major causes of child morbidity mortality in developing countries (Burkih, 1997). Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children less than 5 years of age. World health organization (WHO) estimate that in 1998 (Jaiarj et al., 2000), about 7.1 million deaths occurred due to diarrhoea (Lin et al., 2002). As a result of this, the world health organization (WHO) has set up a diarrhoea control programme (CDD) which include traditional medicine practices (Lutterodt et al., 1999). ). In an effort to tackle the problems of diarrhoea, the World Health Organization (WHO) has established diarrhoea disease control programme (DDC) which includes studies of

traditional medicinal practices together with the evaluation of health education and prevention approaches (WHO, 2014). In most parts of the developing countries, particularly Africa, the use of herbal remedies in management of diarrhoea is a common practice. Some plants have been evaluated for their anti-diarrhoea properties (Mukherejee *et al.*, 1998; Lutterodt, 1992; Morton, 1987). With the emergence of multidrug-resistant organisms, there are few or no treatment options for infections with certain microorganisms [Aemstrong *et al.*, 1995; Wenzel., 2000] as bacteria are resistant to 21 different antibiotics and each isolate is on average resistant to 7–8 antibiotics [D'Costa *et al.*, 2006]. Under these circumstances where treatment becomes challenging, physicians have requested clinical laboratory to assess the adequacy of therapy [DeGirolani, 1987; Isenberg 1988], especially, when bactericidal antimicrobial agent therapy is considered necessary [Peterson 1992].

*Psidium guajava* is a fruit bearing tree commonly known as guava, which belong to the family Myrtaceae. Guava is rich in tannins, phenols, triterpenes, vitamins, fibre and oils, saponins, lectins, vitamins, fibre and fatty acids. Guava fruits is higher in vitamin C than citrus fruits and contains appreciable amount of vitamin A as well. Guava fruits are also a good source of pectin. *Psidium guajava* is useful medicinal plant its leaves have been used in folk medicine for many years to treat diarrhoea, stomach ache and hepatic problems. The bark, leaves, fruit and root have also been evaluated pharmacologically for the treatment of gastrointestinal diseases (Tona,1999). The French call it goyave or goyavier; the Dutch, guyaba, goeajaaba, the Sunninese, guave or goejaba, and the Portuguses, goiaba or goaibeira, Hawaiians call it guava or Kuawa. In Gwam, it is abas. In Malaya, it is generally known either as guava or Jambu batu (Morton, 1987).

Nigerians call it Guava and Igbo part of Nigeria call it Gova, Hausa call it Gwaba, Yoruba call it Gilofa. Guava grows in every states in Nigeria.

*Irvingia gabonensis* is a tree plant popularly known as wild or Africa mango. The plant occurs freely in many parts of Africa. It belongs to the family Irvingiaceae, it is identified by various names such as Bush mango, Dika nut tree, Ugiri in Igbo, Goron or Biri in Hausa and Apon in Yoruba.

Unaeze *et al.*(2017) observed that the inhibitory action of the plant extracts could be attributed to the presence of the phytochemical constituents in the plant extracts such as alkaloid, flavonoid and saponin.

The objective of the study was to evaluate the effect of combining two plants extracts (checkerboard) on different test organisms causing diarrhoea.

## MATERIALS AND METHODS

### Plant collection

*Psidium guajava* and *Irvingia gabonensis* leaves were collected from Lilu town in Ihiala L.G.A of Anambra State, Nigeria. The plants were identified and authenticated in the Department of botany, Nnamdi Azikiwe University, Awka Nigeria where the samples were deposited. The leaves spread out and dried on a clean surface under a shade at room temperature to exclude direct Sunlight in order to prevent the active constituents of the leaves from being degraded due to photochemical reactions. It was air dried for about eight days after which, it was observed to be dried. The dried leaves were gathered, and crushed with grinder. The powders were weighed using an electric weighing balance by Kern ALS 220 – 4. The powders were then stored in an air tight bag at room temperature and used for further extraction.

### Preparation of plant extract

The ground leaves were prepared in three ways to get the extracts.

#### Aqueous extract (*Maceration Method*)

Maceration method was used for aqueous extraction and powdered leaves of *I. gabonensis* and *Psidium guajava* were used. 150 g of the plants were weighed and put in 375 ml of distilled water and allowed to stand for 48 hrs, agitate or shake for 45 mins. The extract was filtered using British standard mesh filter and first muslin cloth and concentrated by using air drying under constant air current and water bath at 50°C. The extract were then transferred into a clean container and stored in the refrigerator until required for use.

#### Organic solvent extraction by maceration

This was carried out at Pharmacognosis Department, Faculty of Pharmaceutical Sciences, Agulu. 150 g of the plants sample were transferred into 1000 ml volumetric flask, then 375 mls of solvent (methanol and n-hexane) were added. This was covered and allowed for 48 hrs with continuous shaking, filtered and transferred to rotary evaporator for concentration. The extracts were then transferred into a clean container and stored in the refrigerator until required for use.

#### Extraction by Soxhlet method

This method was carried out by continuously extracting a sample with a non polar organic solvent for about 4-6 hrs.

#### Control Organisms used for Antimicrobial screening of Plants.

Standard organisms were used for the antimicrobial / antifungal sensitivity testing.

<i>Salmonella typhi</i>	NCTC 10950
<i>E.coli</i>	NCTC 10418
<i>Staphylococcus aureus</i>	NCTC 6571
<i>Candida albican</i>	

Three of these organisms were typed organisms and were obtained from Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical science, Agulu, Nnamdi Azikiwe University. The organisms were sub cultured in different selective media for colony morphology confirmation of the typed organisms. The organisms were re-confirmed through biochemical tests: catalase, coagulase, motility, indole, urease and Triple sugar iron agar (TSI).

These organisms were control organisms and were stored in agar slants in a refrigerator at 2-4°C until used.

Prior to use, these organisms were sub-cultured on Nutrient agar plates, or Sabouraud dextrose agar plates at 37°C for 24 h.

#### Antimicrobial Screening of Plant Extracts

From the stored extracts in the refrigerator, the concentrated aqueous extracts of different plants were weighed 1200 mg of extract (1.2 g) as the stock. The extract was dissolved in 3 mls of distilled water to obtain 400 mg/ml as our interest. This was done for aqueous extracts of the various plants.

1200 mg (1.2 g) of methanol and n-hexane extracts of the plants were weighed and dissolved in 3 mls of DMSO to make a concentration of 400 mg/ml.

#### Determination of Susceptibilities of Organisms to Crude Extracts

Prior to testing, each organism was sub cultured from the nutrient agar slope (storage system) into a nutrient agar plate. This was incubated at 37°C for 24 hrs. After 24 hrs incubation, a colony of each tested organism was inoculated into 5 mls of sterile Nutrient broth and incubated at 37°C for another 24 hrs. Thereafter, turbidity was checked.

The turbidity was adjusted to 0.5 Macfarland standard and diluted to obtain a final turbidity in approximately  $1 \times 10^8$  cfu / ml. The agar diffusion method was employed for this procedure (Lovion, 1980).

Muller Hinton agar was used for bacteria while Sabouraud dextrose agar was used for fungal cultivation. These media were sterilized in an autoclave at 121°C (15 lbs pressure) for 15 min before use. Petri dishes were sterilized in a hot air oven at 175°C for 1 hr and was labelled appropriately.

#### Agar Diffusion Method

From the first concentration (400mg/ml) that was gotten from the stock i.e 1200mg extract dissolved in 3 mls, further doubling dilution was prepared to give 1:200, 1:100, 1:50, 1:25, 1:12.5, 1:6.25, 1:3.125. Then, 0.1ml of broth culture of each tested organism or fungi was placed at the centre of a sterilized petri dish and 20ml of prepared Muller Hinton Agar or Sabouraud's dextrose agar poured into it. The dish was swirled gently to ensure

even distribution of the bacteria or fungi and the mixture was then allowed to gel. When gelled, six wells of 7mm in diameter were bored in each petri dish using a sterile cork borer and each well was labeled appropriately for each crude extract or dilution of crude extract, the wells were carefully filled with 2 drops of a 2ml pipette of both stock solutions(crude extracts) and different dilutions of the extracts, which is equivalent to 0.04 mls starting with the highest dilutions. The plates were kept for 30 mins on the bench for diffusion of the extract to take place before incubation. The dishes were incubated at 37°C for 24 hrs and observed for inhibition. The fungi were inoculated in Sabouraud dextrose agar and incubated at room temperature (25°C) for 24 - 48 hrs. The zones of inhibition were measured and the results noted.

#### Checkerboard Assay

The two plants were interacted to determine the synergistic, indifference or other interactive effect using agar diffusion method. The times two of the individual minimum inhibitory concentration (MIC) were prepared and the two agents were combined in the ratio of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 10:0 ml v/v and were tested against different bacteria and fungi, the combined agents were further diluted using two fold dilution to get graded concentrations of the combinations. The agar diffusion describe above were applied and the preparations were incubated at 37°C and 25°C for 24hrs and 48hrs respectively, thereafter, the MICs of the two agents individually and their MICs in each combination were used to work out the Fractional Inhibitory Concentration (FIC) and finally the FIC index was calculated to determine the nature of the interaction.(okore, 2009).

$$\text{FIC index} = \frac{A^1}{A^{11}} + \frac{B^1}{B^{11}}$$

Where  $A^1$  and  $B^1$  represent MIC of A and B in combination.

While  $A^{11}$  and  $B^{11}$  represent MICs of individual plant extracts.

Synergism ==> when FIC index is less than 1.0

Additivity ==> when FIC index is equal to 1.0

Indifference ==> when FIC index is more than 1.0

Antagonism ==> when FIC index is more than 2.0.

#### RESULTS

Table 1a and 1b shows the MIC and combination effect of different ratios of *Irvingia gabonensis* and *Psidium guajava* leaves extracts at different concentrations against *Staph. aureus* (NCTC 6571) using Checkerboard agar well diffusion method. The plants combination showed synergistic interactions (66.6%), Indifference (33.3%) against the test organism and at different plant ratio, also at ratio of 6:4 the plants had a good synergistic effect, with FIC index of 0.55. The MIC *I. gabonensis* was 12.5 while the MIC of *P. guajava* was 3.12.

Table 2a and 2b shows the MIC and combination effect of different ratios of *I. gabonensis* and *P.guajava* leaves extracts at different concentrations against *Salmonella typhi* (NCTC 10950) using checkerboard agar well diffusion method. Synergistic interaction was most expressed against *S. typhi* (NCTC 10950). The plants combinations showed synergistic interactions (88.8%), Indifference (11. 11%) against the test organism, also at the ratio of 6:4, the plants had a very good synergistic effect, with FIC index of 0.35. The MIC of *I. gabonensis* was 12.5 and the MIC of *P.guajava* was 6.25.

Table 3a and 3b shows the MIC and combination effect of different ratio of *I. gabonensis* and *P.guajava* leaves extracts at different concentrations against *E. coli* (NCTC 10418) using checkerboard agar well diffusion

method. The plants combination showed synergistic interactions (44.44%) and indifference (55.55%) against the test organism, but at the ratio of 6:4 it had a very good synergistic effect with FIC index of 0.33. The MIC of *I. gabonensis* was 12.5, while the MIC of *P. guajava* was 3.13.

Table 4a and 4b shows the MIC and combination effect of different ratios of *I. gabonensis* and *P.guajava* leaves extracts at different concentrations against *Candida albicans* using checkerboard agar well diffusion method. The plants combination showed synergistic interactions (44.44%) and indifference (55.55%) against the test organism, but at the ratio of 6:4 it had synergistic effect with FIC index of 0.70. The MIC of *I. gabonensis* was 12.5, while the MIC of *P. guajava* was 6.25.

**Table 1a: Mic of *Irvingia Gabonensis* and *Psidium Guajava* Ratio of *Salmonella typhi*.**

<i>Salmonella typhi</i> Ratio U(A):G(B)	1 25 : 25 µg: µg	2 12.5:12.5 µg: µg	3 6.25:6.25 µg : µg	4 3.125:3.125 µg : µg	5 1.563:1.563 µg : µg	6 0.7812:0.7812 µg : µg
10 : 0	4	2	+	+	+	+
9 : 1	6	4	2	+	+	+
8 : 2	6	4	2	+	+	+
7 : 3	6	4	2	+	+	+
6 : 4	8	6	4	2	+	+
5 : 5	8	6	4	2	+	+
4 : 6	8	6	4	2	+	+
3 : 7	8	6	4	2	+	+
2 : 8	6	4	2	+	+	+
1 : 9	6	4	2	+	+	+
10 : 0	6	4	2	+	+	+

**Table 1b: Combination of different ratios of *Irvingia gabonensis*(Ugiri) and *Psidium guajava*(Guava) leaves extracts against *salmonella typhi*.**

**Concentrations**

<i>Salmonella typhi</i> Ratio U(A):G(B)	1	2	3	4	5	6	Fic index	Remark
10 : 0	25: 0	2.5: 0	6.25:0	3.13 : 0	1.57: 0	0.79: 0	-	
9 : 1	22.5:2.5	11.25:1.25	3.63:0.63	2.82:0.32	1.41:0.16	0.71:0.08	0.55	Synergism
8 : 2	20: 5.0	10:2.5	5.0:1.25	2.5:0.63	1.25:0.32	0.63:0.16	0.60	Synergism
7 : 3	17.5: 7.5	8.75:3.75	4.38:1.88	2.19:0.94	1.10:0.47	0.55:0.24	1.16	Indifferenc
6 : 4	15:10	7.5:5.0	3.75:2.5	1.88:1.25	0.94:0.79	0.47:0.40	0.35	Synergism
5 : 5	12.5:12.5	6.25:6.25	3.13:3.13	1.57:1.57	0.79:0.79	0.40:0.40	0.38	Synergism
4 : 6	10:15	5.0:7.5	2.5:3.75	1.25:1.88	0.79:0.94	0.40:0.47	0.40	Synergism
3 : 7	7.5:17.5	3.75:8.75	1.88:4.38	0.94:2.19	0.47:1.10	0.24:0.55	0.43	Synergism
2 : 8	5.0:20	2.5:10	1.25:5.0	0.63:2.5	0.32:1.25	0.16:0.63	0.90	Synergism
1 : 9	2.5:22.5	1.25:11.25	0.63:5.63	0.32:2.82	0.16:1.41	0.08:0.71	0.95	Synergism
10 : 0	0 : 25	0 : 12.5	0 : 6.25	0 : 3.12	0 : 1.57	0 : 0.79	-	

A<sup>11</sup> = 12.5, B<sup>11</sup> = 6.25

Table 2a: Mic of *Irvingia Gabonensis* and *Psidium Guajava* Ratio of *E. coli*.

<i>E. coli</i> Ratio U(A):G(B)	1 25 : 25 µg : µg	2 12.5:12.5 µg : µg	3 6.25:6.25 µg : µg	4 3.125:3.125 µg : µg	5 1.563:1.563 µg : µg	6 0.7812:0.7812 µg : µg
10 : 0	4	2	+	+	+	+
9 : 1	4	2	+	+	+	+
8 : 2	6	4	2	+	+	+
7 : 3	8	6	4	2	+	+
6 : 4	10	8	6	4	2	+
5 : 5	6	4	2	+	+	+
4 : 6	6	4	2	+	+	+
3 : 7	8	6	4	2	+	+
2 : 8	6	4	2	+	+	+
1 : 9	6	4	2	+	+	+
10 : 0	8	6	4	2	+	+

Table 2b: Combination of different ratios of *Irvingia gabonensis* and *Psidium guajava* leaves extracts against *E. coli*.

Concentrations

<i>E.coli</i> Ratio U(A):G(B)	1	2	3	4	5	6	Fic index	Remark
10 : 0	25: 0	12.5: 0	6.25:0	3.13 : 0	1.57: 0	0.79: 0	-	
9 : 1	22.5:2.5	11.25:1.25	3.63:0.63	2.82:0.32	1.41:0.16	0.71:0.08	1.30	Indifference
8 : 2	20: 5.0	10:2.5	5.0:1.25	2.5:0.63	1.25:0.32	0.63:0.16	0.79	Synergism
7 : 3	17.5: 7.5	8.75:3.75	4.38:1.88	2.19:0.94	1.10:0.47	0.55:0.24	0.48	Synergism
6 : 4	15:10	7.5:5.0	3.75:2.5	1.88:1.25	0.94:0.79	0.47:0.40	0.33	Synergism
5 : 5	12.5:12.5	6.25:6.25	3.13:3.13	1.57:1.57	0.79:0.79	0.40:0.40	1.25	Indifference
4 : 6	10:15	5.0:7.5	2.5:3.75	1.25:1.88	0.79:0.94	0.40:0.47	1.39	Indifference
3 : 7	7.5:17.5	3.75:8.75	1.88:4.38	0.94:2.19	0.47:1.10	0.24:0.55	0.77	Synergism
2 : 8	5.0:20	2.5:10	1.25:5.0	0.63:2.5	0.32:1.25	0.16:0.63	1.69	Indifference
1 : 9	2.5:22.5	1.25:11.25	0.63:5.63	0.32:2.82	0.16:1.41	0.08:0.71	1.85	Indifference
10 : 0	0 : 25	0 : 12.5	0 : 6.25	0 : 3.12	0 : 1.57	0 : 0.79	-	

A<sup>11</sup> = 12.5, B<sup>11</sup> = 3.13

Table 3a: Mic of *Irvingia Gabonensis* and *Psidium Guajava* Ratio of *Candida albicans*.

<i>Candida albicans</i> Ratio U(A):G(B)	1 25 : 25 µg : µg	2 12.5:12.5 µg : µg	3 6.25:6.25 µg : µg	4 3.125:3.125 µg : µg	5 1.563:1.563 µg : µg	6 0.7812:0.7812 µg : µg
10 : 0	4	2	+	+	+	+
9 : 1	4	2	+	+	+	+
8 : 2	4	2	+	+	+	+
7 : 3	4	2	+	+	+	+
6 : 4	6	4	2	+	+	+
5 : 5	6	4	2	+	+	+
4 : 6	6	4	2	+	+	+
3 : 7	4	2	+	+	+	+
2 : 8	4	2	+	+	+	+
1 : 9	5	3	2	+	+	+
10 : 0	6	4	2	+	+	+

**Table 3b: Combination of different ratios of *Irvingia gabonensis* and *Psidium guajava* leaves extracts against *Candida albicans*.**

**Concentrations**

<i>Candida albicans</i> Ratio U(A):G(B)	1	2	3	4	5	6	Fic index	Remark
10 : 0	25: 0	12.5: 0	6.25:0	3.13 : 0	1.57: 0	0.79: 0	-	
9 : 1	22.5:2.5	11.25:1.25	3.63:0.63	2.82:0.32	1.41:0.16	0.71:0.08	1.10	Indifference
8 : 2	20: 5.0	10:2.5	5.0:1.25	2.5:0.63	1.25:0.32	0.63:0.16	1.20	Indifference
7 : 3	17.5: 7.5	8.75:3.75	4.38:1.88	2.19:0.94	1.10:0.47	0.55:0.24	1.62	Indifference
6 : 4	15:10	7.5:5.0	3.75:2.5	1.88:1.25	0.94:0.79	0.47:0.40	0.70	Synergism
5 : 5	12.5:12.5	6.25:6.25	3.13:3.13	1.57:1.57	0.79:0.79	0.40:0.40	0.75	Synergism
4 : 6	10:15	5.0:7.5	2.5:3.75	1.25:1.88	0.79:0.94	0.40:0.47	0.80	Synergism
3 : 7	7.5:17.5	3.75:8.75	1.88:4.38	0.94:2.19	0.47:1.10	0.24:0.55	1.70	Indifference
2 : 8	5.0:20	2.5:10	1.25:5.0	0.63:2.5	0.32:1.25	0.16:0.63	1.78	Indifference
1 : 9	2.5:22.5	1.25:11.25	0.63:5.63	0.32:2.82	0.16:1.41	0.08:0.71	0.95	Synergism
10 : 0	0 : 25	0 : 12.5	0 : 6.25	0 : 3.12	0 : 1.57	0 : 0.79	-	

$A^{11} = 12.5, B^{11} = 6.25$

Key:

U = Ugiri (A).

G = Guava (B)

**Table 4a: Mic of *Irvingia Gabonensis* and *Psidium Guajava* Ratio of *Staph. aureus*.**

<i>Staph. aureus</i> Ratio U(A):G(B)	1 25 : 25 µg : µg	2 12.5:12.5 µg : µg	3 6.25:6.25 µg : µg	4 3.125:3.125 µg : µg	5 1.563:1.563 µg : µg	6 0.7812:0.7812 µg : µg
10 : 0	4	2	+	+	+	+
9 : 1	4	2	6	+	+	+
8 : 2	5	3	1	+	+	+
7 : 3	6	4	2	+	+	+
6 : 4	8	6	4	2	+	+
5 : 5	8	6	4	2	+	+
4 : 6	8	6	4	2	+	+
3 : 7	6	4	2	+	+	+
2 : 8	6	4	2	+	+	+
1 : 9	8	6	4	2	+	+
10 : 0	8	6	4	2	+	+

**Table 4b: Combination of different ratios of *Irvingia gabonensis* and *Psidium guajava* leaves extracts against *s. aureus*.**

**concentrations**

<i>S. aureus</i> Ratio U(A):G(B)	1	2	3	4	5	6	Fic index	Remark
10 : 0	25 : 0	12.5 : 0	6.25 : 0	3.13 : 0	1.57 : 0	0.79 : 0	-	
9 : 1	22.5:2.5	11.25:1.25	5.63:0.63	2.82:0.32	1.41:0.16	0.71:0.08	1.30	Indifference
8 : 2	20 : 5	10 : 2.5	5 : 1.25	2.5 : 0.63	1.25:0.32	0.63:0.16	0.80	Synergism
7 : 3	17.5 : 7.5	8.75 : 3.75	4.38:1.88	2.19:0.94	1.10:0.47	0.55:0.24	0.95	Synergism
6 : 4	15 : 10	7.4 : 5	3.75:2.5	1.88:1.25	0.94:0.79	0.47:0.40	0.55	Synergism
5 : 5	12.5:12.5	6.25 : 6.25	3.13:3.13	1.57:1.57	0.79:0.79	0.40:0.40	0.63	Synergism
4 : 6	10 : 15	5 : 7.5	2.5 : 3.75	1.25:1.88	0.79:0.94	0.40:0.47	0.70	Synergism
3 : 7	7.5 : 17.5	3.75 : 8.75	1.88:4.38	0.94:2.19	0.47:1.10	0.24:0.55	1.55	Indifference
2 : 8	5 : 20	2.5 : 10	1.25 : 5	0.63:2.5	0.32:1.25	0.16:0.63	1.70	Indifference
1 : 9	2.5 : 22.5	1.25 : 11.25	0.63 : 5.63	0.32 : 2.82	0.16 : 1.41	0.08 : 0.71	0.93	Synergism
10 : 0	0 : 25	0 : 12.5	0 : 6.25	0 : 3.12	0 : 1.57	0 : 0.79	-	

$A^{11} = 12.5, B^{11} = 3.12$

Key:

U = Ugiri (A).

G = Guava (B)

## DISCUSSION

The results of the present study indicated that the combination of *I. gabonensis* and *P. guajava* leaves had significant synergistic effects on all the tested organisms (*Staph. aureus*, *Salmonella typhi*, *E. coli* and *Candida albicans*) but had the highest synergistic effect on *Salmonella typhi* at different ratios. Moreso, the overall synergistic effect of the interactions are more than the indifference effect. The interaction between the two plants at different ratios generally enhanced the activity against the organisms. Giordani et al, 1997 had similar result. The in vitro activities of the extracts combinations were further assessed on the basis of the fractional inhibitory concentration (Fic) index representing the sum of the  $FIC_s$  ( $\sum FIC_s$ ) of each plant tested, where the FIC is determined for each plant by dividing the MIC of each plant when used in combination by the MIC of each plant when used alone. The FIC values obtained by checkerboard assay showed less than 1 in combination, this indicating synergistic interaction between *I. gabonensis* and *P. guajava*. Moreso, unaeze et al 2017, also suggested that the two plants had anti- diarrhoea effect.

## CONCLUSION

The combination of plant extracts demonstrating *in vitro* synergism against infectious agents are most likely to be a means of achieving pragmatic and effective treatment for bacterial and fungal infections especially in patients with infections difficult to treat. The study suggest that the combination of two plants could perform better in the management of diarrhoea than monotherapy.

## COMPETING INTERESTS

The authors declare that there are no conflicts of interest.

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