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Effect of Plant Extract Combinations on Some Bacterial Pathogens

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ABSTRACT: Increase in antimicrobial resistance coupled with successful treatment of various diseases with herbal medications has triggered the upsurge in research geared towards harnessing the medicinal potentials of various plants. The aim of this study was to evaluate the phytochemical composition *of Bryophyllum pinnatum, Ocimum gratissimum, Jatropha curcas* and *Ficus exasperata* and their combined antibacterial activity on *Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Klebsiella pneumonia*. Plant leaves were air–dried, extracted with ethyl-acetate and water with subsequent qualitative analyses for phytochemical compounds. Bacterial pathogens were obtained from University of Benin Teaching Hospital (UBTH), confirmed, and their zones of inhibition studied at 50, 40, 30, 20 and 10 mg/ml of extract combinations. The presence of phenols, tannins and saponins were confirmed in all extracts while alkaloids were present in all ethyl acetate extracts and terpenoids in all aqueous extracts. Different zones of inhibition were measured with the different extract combinations with *E. coli* having its highest zone of inhibition with the combination of *Bryophyllum pinnatum* and *Ocimum gratissimum* in 30 mg/ml (27.25 ± 1.70 mm). However, the highest zone of inhibition observed in the study was with *S. aureus* in 10 mg/ml concentration of ethyl acetate plant extracts of *Ocimum gratissimum* and *Ficus exasperata* (31.75 ± 3.07 mm). The Gram positive cell wall of *S. aureus* is less complex and therefore, more susceptible to bio-agents. Antibacterial activities of these extracts are attributed to the presence of secondary metabolites that make them good bio-agents for production of antibacterial drugs.

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Pharmacological industries have been left with the challenge of occasional emergence of bacterial strains with the ability to overcome the effect of antibiotics previously used to treat them (Odey *et al*., 2012; Awala *et al*., 2017). Resistance to these drugs by bacteria has resulted in the need to source for alternative antibacterial agents such as plant extracts (WHO, 2000; Costa *et al*., 2015; Ildiz *et al*., 2018). Plants of medicinal importance contain huge varieties of phytochemicals which possess important therapeutic properties that can be used for the treatment of emerging and re-emerging human diseases (Abbas *et al*., 2017; Tyagi *et al*., 2017; Kin *et al*., 2018). Phytochemical substances such as alkaloids, essential oils, peptides, tannins, phenols and flavonoids are medicinal agents responsible for the antimicrobial ability of the plants especially against disease causing microorganisms (Odey *et al*., 2012; Costa *et al*., 2015; Udochukwu *et al*., 2015; Rukmini *et al*., 2017; Aggarwal *et al*., 2017).

Leaf extracts of *Occimium gratissimum* (scent leaf), *Fiscus exasperata Vahl* (sand paper tree), *Jathropha curcas* (purging nut) and *Bryophyllum pinnatum* (miracle plant) have been singly analyzed as natural antimicrobials against various pathogenic organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus fecalis* and *Pseudomonas aeruginosa* (Terezinha *et al*., 2008; Akinjogunla *et al*., 2009; Ladipo *et al*., 2010. The efficacy of these medicinal plants however, depends on their solubility in solvents of different polarity (Odey *et al*., 2012; Zlotek *et al*., 2016). Plants antimicrobials have been found to be synergistic enhancers in that though they may have small antimicrobial properties alone, when taken concurrently with other plants enhance these effects (Rakholiya and Chanda, 2012). In this regard, this study aimed at analyzing the phytochemical extracts of *Occimium gratissimum*, *Ficus exasperata, Jathropha curcas* and *Bryophyllum pinnatum* and their combined antibacterial activities against selected human bacterial pathogens.

MATERIALS AND METHODS

Collection of Plant Materials: The fresh leaves of *Bryophyllum pinnatum, Ocimum gratissimum, Jatropha curcas* and *Ficus exasperata* were collected from Sapele road, Benin City, Nigeria. The plants

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were identified and authenticated by the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

Preparation of plant materials: The fresh leaves were rinsed and shade dried to a constant weight over a period of time. The dried leaves were pulverized using British mechanical grinder. The powdered leaves weighed 150 g per plant and were soaked separately in 500 ml of ethyl-acetate and aqua for 72 hours using cold method of maceration. The macerated materials were filtered, and the filtrate concentrated to dryness under reduced temperature in water bath using crucibles. The dried extracts were stored in a separate air-tight clean glass container at 4 ^{o}C for further use.

Collection of bacterial pathogens: Staphylococcus aureus, Escherichia coli, Salmonella typhi and *Klebsiella pneumonia* were obtained from University of Benin Teaching Hospital (UBTH) and were put in slants and stored in a refrigerator until use. The isolates were confirmed in the Department of Microbiology, University of Benin

Qualitative phytochemical analyses of plants extracts: Standard procedures were used to detect and quantify flavonoids, alkaloids, phlobatannins, phenolic compounds, tannins, saponins, cardiac glycosides, and terpenoids compounds in the four plants extracts (Harborne, 1973; Obadoni and Ochuko, 2001)

Preparation of Extract Combinations: The four plant leaves of *Bryophyllum pinnatum* (B)*, Ocimum gratissimum* (O)*, Jatropha curcas* (J) and *Ficus exasperata* (F) extracts of ethyl-acetate and aqua made up to eight extracts which were combined separately in twos, threes and a four in five concentrations of 50, 40, 30, 20 and 10 mg/ml. These combinations in twos were BO, BJ, BF, OJ, OF, JF; in threes they were BOJ, BOF, BJF, OJF and in four was BOJF.

Antibacterial Activity of different combined Extracts: The mixtures of the different dissolved plant extracts combinations were stirred with a sterile glass rod and left to stand for 20 minutes. Discs (sterilized Whatmann no. 1 filter paper cut with a hand paper-punch) were introduced into the mixture, and were left to soak for 2 hours before introducing them in plates containing the bacterial pathogens.

Antibacterial Assay: Pathogenic bacterial isolates growing on nutrient agar plates were picked to make suspensions in 1ml sterile normal saline and this was adjusted to an equivalence of a 0.5 McFarland standard. Sterile Mueller-Hinton agar (Oxoid, UK) plates were inoculated by spreading 0.1ml of each bacteria inoculum suspension on the entire surface of the plate and the extracts impregnated discs were inserted. Inoculated plates with discs impregnated with crude extracts were placed in an incubator at 37 ^{o}C for 24 hours. The zones of inhibition were measured using a ruler to the nearest millimetre and recorded.

Statistical Analysis: Zones of inhibition (mm) of the different plants extracts combinations were done in replicates and means with standard errors were calculated using the Statistical Package for Social Sciences software (SPSS version 20). Hypothesis testing using t-Test was conducted at $p < 0.05$ confidence level (Ogbeibu, 2014).

RESULTS AND DISCUSSION

Four different plant leaves of *Bryophyllum pinnatum, Ocimum gratissimum, Jatropha curcas* and *Ficus exasperata* were extracted with both Ethyl acetate and aqua, and their phytochemical contents analyzed. Both extractants pooled out tannins, saponins and phenols from all plants leaves (Tables 1 and 2).This was not surprising because plant extracts contain metabolites such as phenolics, terpenes and alkaloids that show both biological and pharmacological activities (Stefanovic and Comic, 2012; Kin *et al*., 2018). However, alkaloids were only present in plant leaves extracted with Ethyl acetate and in *Ficus exasperata* in the aqueous extract. Similarly, Awala *et al*. (2017) showed the presence of phenols, tannins and alkaloids in acetone and methanol extracted leaves of *Ficus exasperata*. Conversely, aqueous extracts of all plants leaves contained terpenoids but this was only observed in *B. pinnatum* extracted in Ethyl acetate (Tables 1 and 2). Kin *et al*. (2018) similarly reported the absence of terpenoids in methanol extracted *O. gratissimum*. *Bryophyllum pinnatum* (B)*, Ocimum gratissimum* (O)*, Jatropha curcas* (J) and *Ficus exasperata* (F) Ethyl acetate and aqua leaves extracts at 50, 40, 30, 20 and 10 mg/ml concentrations were combined in twos, threes, and a four and their antibacterial activities were assessed (Tables 3 and 4). Zones of inhibition on bacterial lawn incorporated with plant extracts discs were used to measure antibacterial activities. For *E. coli*, the highest zones of inhibition measured where in the two combinations of *Bryophyllum pinnatum* and *Ocimum gratissimum* in 30 mg/ml (27.25 ± 1.70 mm) and the four combinations of *Bryophyllum pinnatum, Ocimum gratissimum, Jatropha curcas* and *Ficus exasperata* (26.50 ± 1.55) in 10 mg/ml using Ethyl acetate (Table 3). Similarly, aqua extracts of *Bryophyllum pinnatum, Ocimum gratissimum* and *Jatropha curcas* in 30 mg/ml (20.00 ± 1.78) had the highest zone of inhibition.

Table 1: Qualitative phytochemical constituent of Ethyl acetate extracts across various plants

Phytochemicals	Plant Extracts			
	B. pinnatum	*O. gratissimum*	*J. curcas*	*F. exerspifolia*
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Phlobatannins	-	-	-	-
Saponins	+	+	+	+
Terpenoids	+	-	-	-
Cardiac glycosides	+	+	+	+
Phenols	+	+	+	+

Keys: *+ indicates present – indicates absent*

Table 2: Qualitative phytochemical constituent of Aqueous extracts across various plants

Phytochemicals	Plant Extracts			
	B. pinnatum	*O. grattissimum*	*J. curcas*	*F. exasperata*
Alkaloids	-	-	-	+
Flavonoids	+	-	+	+
Tannins	+	+	+	+
Phlobatannins	-	+	+	-
Saponins	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	-	+	-	+
Phenols	+	+	+	+

Keys: *+ indicates present – indicates absent*

These observed zones of inhibition are as a result of secondary metabolites in the leave extracts such as alkaloids, flavonoids, phenols and essential oils which affect bacterial sensitivity to medicinal plants (Dawoud *et al*., 2013; Ildiz et al., 2018; Kin *et al*., 2018). Higher zones of bacterial inhibition was observed in this study as compared to that of *E. coli, P. aeruginosa* and *S. aureus* at 100 µl/ml (7.0 - 11.5 mm) reported with ethanol and aqueous extracts of *O. gratissimum* (Udochukwu *et al*., 2015). This is likely as a result of combined effects of the plant extracts in relation to only a single extract. Plants extracts may interact with one another to improve their solubility, and thereby enhance their bioavailability and subsequent antibacterial activity (Chouhan *et al*., 2017). However, combinations of BJF and OJ at lower concentrations using Acetyl acetate were not very effective in inhibiting *E.coli*. This could be as a result of lower concentrations of the phytochemicals at these extract combinations. The highest zone of inhibition observed in *S. enterica* when Ethyl acetate plants extracts were introduced was with BOF (27.00 ± 1.23 mm) combinations in 20 mg/ml concentration while this was in 30 mg/ml in BOJ (30.00 ± 3.54) combination using aqueous extract (Table 4). Three combinations of the plant extracts had better antibacterial activity against *S. enterica* as compared to the twos and the four combinations. Ildiz *et al.* (2018) showed that the combination of cefoxitin with coriander extracts had stronger effect than cefoxitin alone on Methicillin Resistant Staphylococcus aureus (MRSA), *E.coli* and *K. pneumonia*. The different combinations of plant extracts showed varied zones of antibacterial activity against *K. pneumonia*. However, the highest zones of inhibition were observed in 20 mg/ml concentration of both Acetyl acetate and aqueous extracts. Although this was with three combinations of BOF (20.00 ± 3.54) in the former and in two combinations of BF (26.25 ± 2.39) in the later. This is similar to the work of Ildiz *et al*. (2018) where 25 mm zone of inhibition of *K. pneumoniae* with petroleum ether extracted coriander seed was reported. Interestingly, the highest antibacterial activity observed in the study was against *S. aureus* and this was observed in 10 mg/ml concentration of Ethyl acetate plant extracts of OF (31.75 ± 3.07) and in 50 mg/ml of BOJF (30.00 ± 0.00) aqueous extract. This agrees with the findings of Awala *et al*. (2017) who reported 31.27 mm zone of inhibition of *Ficus exasperata* acetone and methanol extract against *S. aureus*. The high antibacterial activity against *S. aureus* could be because of the Gram positive cell wall which is less complex and more susceptible to bioagents as compared to that of the Gram negative bacteria (*E. coli, S. typhi, S.* and *K. pneumonia*). Several authors have reported that plant extracts can act synergistically against bacterial pathogens of public health importance (Rojas *et al*., 2004; Prakash *et al*., 2006; Karmegam *et al*., 2008; D'Arrigo *et al*., 2010). Combined effect of ethanolic leaf extracts of *Catharanthus roseus, Lawsonia inermis* and *Chrysanthemum odoratum* exerted a higher activity against MRSA as compared to single leaf extracts. On the whole, the leave extracts in this study are versatile in bioactive agents and demonstrate antibacterial activities against the studied human pathogens.

Table 3: Synergistic effect of Ethyl acetate extracts of plants against selected bacterial pathogens

Pathogens	Conc. (mg/ml)	Zone of Inhibition (mm) of Extract Combinations										
		BO	BJ	BF	BOJ	BOF	BJF	BOJF	OJ	OF	OJF	JF
E. coli	50	10.25±0.25^{b}	13.25±0.63^{e}	10.75±0.48^{b}	12.50±0.65^{d}	12.00±0.41^{c}	8.75±0.48^{a}	10.25±0.25^{b}	11.25±0.63^{b}	10.00±0.00^{a}	9.75±0.25^{a}	11.25±0.63^{b}
	40	15.25±0.63^{d}	11.00±0.00^{c}	10.50±0.29^{b}	10.50±0.50^{b}	10.25±0.25^{b}	0.00±0.00^{a}	9.50±0.29^{b}	18.25±0.63^{e}	9.50±0.29^{b}	15.75±0.63^{d}	11.00±0.29^{c}
	30	27.25±1.70^{f}	10.75±0.48^{b}	10.00±0.41^{b}	11.25±0.95^{b}	17.00±1.23^{e}	0.00±0.00^{a}	10.00±0.00^{b}	0.00±0.00^{a}	12.75±0.48^{c}	13.50±1.04^{d}	10.75±0.48^{b}
	20	14.00±0.82^{d}	9.50±0.29^{b}	9.75±0.48^{b}	8.25±2.43^{b}	10.00±0.00^{b}	0.00±0.00^{a}	10.75±0.48^{b}	0.00±0.00^{a}	8.50±0.85^{b}	12.75±0.25^{c}	9.75±0.25^{b}
	10	9.50±0.29^{b}	8.50±0.29^{b}	8.25±0.25^{b}	13.50±0.96^{d}	13.00±0.58^{d}	0.00±0.00^{a}	26.50±1.55^{e}	0.00±0.00^{a}	9.50±0.29^{b}	11.00±0.41^{c}	10.75±0.25^{c}
S. enterica	50	11.75±0.48^{a}	11.25±0.75^{a}	9.50±0.65^{a}	12.75±0.75^{b}	18.75±1.44^{c}	12.75±0.25^{b}	12.75±0.48^{b}	12.50±0.50^{b}	17.00±1.23^{c}	10.00±0.41^{a}	10.75±0.48^{a}
	40	14.00±0.41^{d}	13.75±0.75^{c}	10.75±0.75^{a}	12.75±0.25^{b}	18.75±1.89^{e}	10.75±0.48^{a}	9.50±0.29^{a}	11.25±0.25^{a}	12.25±1.11^{b}	10.25±0.63^{a}	9.25±0.48^{a}
	30	17.50±1.19^{g}	11.50±1.04^{b}	14.50±0.50^{e}	17.25±1.60^{g}	12.25±0.25^{c}	0.00±0.00^{a}	10.25±0.25^{b}	16.50±0.96^{f}	10.25±0.25^{b}	14.00±1.00^{d}	9.25±0.48^{b}
	20	15.25±2.06^{d}	9.75±0.25^{a}	10.00±0.00^{a}	11.25±0.48^{a}	27.00±1.23^{e}	8.75±0.25^{a}	11.50±0.29^{b}	13.00±0.82^{c}	9.25±0.25^{a}	11.00±0.71^{a}	10.25±0.25^{a}
	10	9.25±0.25^{b}	4.00±2.31^{a}	9.75±0.25^{b}	11.50±0.87^{b}	16.75±1.18^{c}	9.25±0.48^{b}	15.00±0.00^{c}	10.25±0.25^{b}	9.75±0.25^{b}	10.50±0.65^{b}	10.75±0.48^{b}
K pneumoniae	50	10.00±0.00^{a}	10.25±0.25^{a}	9.75±0.25^{a}	10.00±0.00^{a}	14.75±0.25^{d}	10.75±0.48^{a}	11.25±0.48^{b}	10.00±0.41^{a}	10.75±0.25^{a}	12.25±0.95^{c}	12.25±0.63^{c}
	40	9.25±0.25^{a}	9.75±0.25^{b}	9.50±0.48^{b}	11.25±0.41^{d}	11.50±0.48^{d}	8.25±0.48^{a}	9.25±0.41^{a}	10.00±0.48^{b}	10.00±0.00^{b}	10.75±0.48^{c}	10.00±0.41^{b}
	30	8.75±0.48^{a}	7.75±0.28^{a}	16.75±1.03^{f}	14.00±0.00^{e}	12.75±0.25^{d}	9.25±0.25^{a}	8.50±0.28^{a}	11.50±1.19^{c}	8.50±0.29^{a}	10.00±0.41^{b}	10.00±0.00^{b}
	20	7.50±0.29^{b}	8.00±0.41^{b}	9.25±0.25^{b}	12.00±0.41^{c}	20.00±3.54^{d}	0.00±0.00^{a}	8.50±0.29^{a}	10.00±0.41^{b}	8.00±0.41^{b}	10.75±0.48^{b}	9.50±0.29^{b}
	10	8.75±0.25^{b}	7.75±0.25^{b}	7.50±0.29^{b}	13.25±1.03^{e}	12.25±0.95^{d}	0.00±0.00^{a}	8.25±0.48^{b}	16.25±1.32^{f}	7.75±0.25^{b}	10.75±0.75^{c}	9.25±0.25^{b}
S. aureus	50	12.25±0.95^{e}	9.00±0.41^{a}	10.00±0.00^{a}	12.00±0.00^{d}	12.50±0.87^{e}	11.25±0.48^{c}	13.25±0.63^{f}	10.75±0.75^{b}	8.50±0.65^{a}	11.00±0.71^{c}	10.25±0.25^{a}
	40	21.50±0.96^{d}	10.00±0.41^{a}	11.00±0.41^{a}	11.50±0.29^{a}	13.50±1.19^{b}	11.75±0.63^{a}	16.75±1.32^{c}	10.25±0.25^{a}	10.00±0.00^{a}	16.00±1.41^{c}	10.25±0.48^{a}
	30	23.00±1.73^{d}	9.75±0.25^{a}	9.00±0.00^{a}	14.25±0.25^{b}	14.50±0.29^{b}	9.75±0.25^{a}	18.00±0.71^{c}	11.25±0.95^{a}	18.75±0.75^{c}	9.75±0.25^{a}	9.75±0.25^{a}
	20	22.50±1.50^{c}	9.00±0.00^{a}	11.25±0.25^{a}	10.75±0.25^{a}	19.75±1.93^{b}	10.00±0.41^{a}	25.00±0.00^{d}	11.50±0.29^{a}	10.25±0.25^{a}	9.25±0.25^{a}	9.25±0.25^{a}
	10	14.00±0.71^{c}	8.50±0.29^{a}	10.00±0.41^{a}	10.00±0.00^{a}	10.50±0.29^{a}	12.00±0.58^{a}	13.25±0.63^{b}	22.00±1.78^{d}	31.75±3.07^{e}	11.25±0.63^{a}	8.25±0.25^{a}

Key: B: *Bryophyllum pinnatum*; O: *Ocimum gratissimum*; J: *Jathropha curcas*; F: *Ficus experata;* a-f: different characters in the same row indicate values with significant difference (p<0.05)

Table 4: Synergistic effect of aqueous extracts of plants against selected bacterial pathogens

Pathogens	Conc. (mg/ml)	Zone of Inhibition (mm) of Extract Combinations										
		BO	BJ	BF	BOJ	BOF	BJF	BOJF	OJ	OF	OJF	JF
E. coli	50	10.25±0.48^{a}	9.25±0.48^{a}	12.25±0.95^{c}	13.00±0.71^{d}	12.00±0.00^{b}	10.50±0.29^{a}	10.75±0.48^{a}	10.75±0.25^{a}	11.25±0.25^{b}	11.00±0.91^{a}	10.25±0.25^{a}
	40	13.25±0.63^{c}	6.75±0.25^{a}	11.25±0.75^{b}	11.25±0.25^{b}	14.00±0.71^{d}	17.00±1.23^{e}	9.75±0.48^{b}	13.75±0.48^{d}	10.00±0.00^{b}	12.75±1.11^{c}	9.75±0.48^{b}
	30	11.75±0.75^{c}	6.25±0.25^{a}	10.50±0.65^{b}	20.00±1.78^{e}	10.75±0.75^{b}	16.00±1.35^{d}	9.50±0.29^{b}	10.50±0.29^{b}	9.25±0.25^{b}	9.75±0.25^{b}	8.75±0.48^{b}
	20	13.75±0.75^{d}	10.75±0.48^{b}	9.75±0.48^{a}	13.75±0.63^{d}	12.25±0.75^{c}	14.25±1.44^{d}	8.50±0.29^{a}	8.00±0.00^{a}	8.75±0.48^{a}	9.50±0.65^{a}	13.00±0.91^{d}
	10	10.25±0.25^{b}	9.00±0.00^{b}	9.00±0.00^{b}	15.75±2.72^{d}	13.25±1.03^{c}	11.25±0.95^{b}	10.00±0.00^{b}	0.00±0.00^{a}	10.25±0.25^{b}	11.25±0.63^{b}	18.75±1.49^{e}
S. enterica	50	10.00±0.00^{b}	0.00±0.00^{a}	11.75±0.25^{c}	18.75±0.75^{d}	12.00±0.41^{c}	11.75±1.11^{c}	18.25±1.18^{d}	17.25±1.32^{d}	11.75±0.25^{c}	11.50±0.96^{c}	9.00±0.71^{b}
	40	9.25±0.25^{b}	0.00±0.00^{a}	10.00±0.41^{b}	15.25±0.48^{e}	12.50±0.65^{d}	15.75±1.44^{e}	16.75±1.18^{e}	9.00±0.41^{b}	10.50±0.29^{c}	11.00±0.71^{c}	8.00±0.41^{b}
	30	8.75±0.48^{a}	13.25±0.63^{a}	10.50±0.29^{a}	30.00±3.54^{d}	20.50±1.66^{c}	15.00±0.91^{b}	14.25±0.25^{b}	11.50±0.96^{a}	12.00±0.71^{a}	8.75±0.48^{a}	26.75±2.46^{d}
	20	7.50±0.29^{a}	9.25±0.25^{a}	13.25±0.63^{d}	12.50±0.65^{d}	14.00±0.91^{d}	10.00±0.00^{b}	14.25±0.25^{d}	10.00±0.00^{b}	18.25±1.18^{e}	12.25±1.25^{c}	23.00±1.35^{e}
	10	8.75±0.25^{a}	8.75±0.48^{a}	9.25±0.25^{a}	9.75±0.25^{a}	26.00±1.00^{d}	9.50±0.50^{a}	12.75±0.75^{b}	8.75±0.25^{a}	18.75±1.25^{c}	10.25±0.25^{a}	18.75±0.75^{c}
K pneumoniae	50	11.25±0.48^{c}	9.75±0.25^{a}	12.50±0.87^{c}	13.75±0.48^{f}	12.00±1.00^{d}	16.75±1.18^{g}	13.00±0.41^{e}	9.00±0.58^{a}	10.00±0.00^{a}	11.00±0.71^{b}	8.50±0.29^{a}
	40	18.25±1.18^{e}	8.75±0.48^{a}	12.50±1.04^{c}	11.75±0.25^{c}	10.50±0.29^{a}	15.00±1.47^{d}	11.00±0.41^{c}	9.00±0.00^{a}	9.00±0.00^{a}	16.00±1.41^{e}	8.25±0.48^{a}
	30	6.75±0.48^{a}	9.00±0.71^{b}	10.25±0.25^{b}	17.00±1.47^{d}	10.00±0.00^{b}	15.25±0.85^{c}	10.50±0.50^{b}	10.50±0.29^{b}	13.25±1.32^{c}	9.50±0.29^{b}	9.00±0.00^{b}
	20	8.75±0.25^{a}	9.00±0.41^{a}	26.25±2.39^{d}	15.25±0.48^{c}	9.50±0.50^{a}	10.50±0.87^{a}	13.00±0.71^{b}	11.25±0.25^{a}	9.75±0.48^{a}	9.25±0.25^{a}	10.00±0.00^{a}
	10	10.00±0.00^{b}	9.75±0.48^{b}	11.50±0.96^{b}	17.25±1.32^{c}	7.75±0.25^{a}	10.75±0.85^{b}	15.50±0.87^{c}	10.25±0.25^{b}	10.50±0.50^{b}	11.25±0.63^{b}	7.50±0.29^{a}
S. aureus	50	12.00±0.58^{a}	10.75±0.25^{a}	11.25±0.48^{a}	13.75±0.48^{b}	21.25±2.39^{c}	19.50±0.87^{c}	30.00±0.00^{d}	13.00±0.71^{a}	11.75±0.48^{a}	11.00±0.41^{a}	11.25±0.48^{a}
	40	14.50±0.50^{b}	9.50±0.29^{a}	23.50±0.96^{f}	11.25±0.25^{a}	13.75±0.48^{b}	17.75±1.25^{c}	12.00±0.00^{a}	11.25±0.48^{a}	18.50±1.26^{d}	21.50±3.01^{e}	9.75±0.25^{a}
	30	13.75±1.03^{b}	9.75±0.25^{a}	20.00±1.23^{e}	13.75±0.48^{b}	15.75±3.09^{c}	17.50±1.66^{d}	14.25±0.25^{b}	9.75±0.48^{a}	12.25±0.48^{a}	11.25±0.63^{a}	14.25±0.48^{b}
	20	10.50±0.29^{a}	10.00±0.41^{a}	20.75±1.70^{d}	16.25±1.25^{c}	13.25±0.25^{b}	16.75±1.44^{c}	15.75±0.48^{c}	11.75±0.75^{a}	9.50±0.50^{a}	11.25±0.25^{a}	10.75±0.25^{a}
	10	8.00±0.00^{a}	8.25±0.25^{a}	13.75±0.48^{b}	17.25±1.03^{c}	19.25±0.75^{c}	13.00±1.16^{b}	20.75±1.11^{d}	13.25±1.11^{b}	8.00±0.41^{a}	10.25±0.48^{a}	21.75±1.18^{e}

Key: B: *Bryophyllum pinnatum*; O: *Ocimum gratissimum*; J: *Jathropha curcas*; F: *Ficus experata;* a-f: different characters in the same row indicate values with significant difference (p<0.05)

Conclusion: Phytochemicals present in the leave extracts of *B. pinnatum, O. graticcimum, J. curcas* and *F. exasperate* enhanced their antibacterial activities in combinations. *S. aureus* and *E. coli* had the highest zones of inhibition observed in the study with a combination of *Ocimum gratissimum* and *Ficus exasperate* for *S. aureus*, and *Bryophyllum pinnatum* and *Ocimum gratissimum* for *E. coli.* Studies on combined plant extracts interactions enhance their bioavailability and antibacterial activity as compared to studies on single extracts. Therefore, they can be good sources of antibacterial drugs.

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