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Evaluation Of The Potencies Of Gongronema Latifolium, Chromolaena Odorata And Ocimum Gratissimum Leaf Extracts As Herbal Alternatives For Management Of Puerperal Sepsis: An Animal Model Investigation

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Abstract
Puerperal sepsis and its associated complications remain a major cause of maternal mortality especially in Africa and countries with struggling economies, and contributes significantly to increasing global disease burden. In this study, the potencies of Gongronema latifolium, Chromolaena odorata and Ocimum gratissimum leaf extracts in the management of Streptococcus agalactiae-induced puerperal sepsis were investigated in rats. Extracts prepared from the dried plant materials were subjected to phytochemical analysis, acute toxicity test and in vitro antimicrobial evaluations. In the puerperal study, 40 pregnant rats assigned to 8 groups of 5 rats were used. Group 1 was the normal control but puerperal sepsis was induced in groups 2 to 8 via intravaginal inoculation with Streptococcus agalactiae a day after delivery and thereafter treated with graded dose levels of the extracts with group 2 remaining untreated and served as the puerperal control. Treatment lasted for 21 days before the rats were sacrificed and blood and uterine samples collected for haematological, serum biochemical and histopathological analyses. Results obtained showed that of the 3 plant materials, Chromolaena odorata had higher yield (4.13%) than Ocimum gratissimum (3.06%) and Gongronema latifolium (2.81%) but all the extracts had significant amounts of phytochemicals (alkaloids, flavonoids, terpenoids, steroids, tannins, cardiac glycosides and saponins) and lethal dose values higher than 5000 mg/kg body weight. The extracts also had significant antimicrobial activities against some bacterial and fungal isolates including Streptococcus agalactiae. In the puerperal rats, the extracts significantly improved body weight gains, reduced pyrexia, lowered pain reflexes and ameliorated observed changes in haematological parameters in the treated infected rats when compared with the puerperal control ($p<0.05$). Elevated serum concentrations of inflammatory cytokines (interleukin 1beta, interleukin-6 and tumor necrosis factor alpha) were all significantly lowered following treatment with the extracts. Histopathological evaluations of the harvested uterine tissues showed significantly reduced infiltration of inflammatory cells and degenerations in the extracts treated rats when compared with the puerperal control. Comparative evaluation of the extracts showed better anti-puerperal activities for Gongronema latifolium and Ocimum gratissimum leaf extracts, hence the conclusion that these two may be safe agents for the management of puerperal sepsis and its associated haematological and biochemical complications.
Keywords: *Antimicrobial, body weights, extracts, phytochemical, puerperal sepsis,*

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I. Introduction

Puerperal sepsis, an infection of the genital tract which occurs at labour or within 42 days of delivery has long been recognized as a major source of concern in maternal health and a major contributor to the increasing prevalence of postpartum complications and death amongst women (Ali *et al.,* 2025). The World Health Organisation (WHO) had at a time reported that out of an estimated 358,000 maternal deaths which occurred yearly due to child birth complications, up to 15% are directly associated with puerperal sepsis (WHO, 2010). In the same year, puerperal sepsis was fingered as the second leading cause of maternal deaths in Nigeria, as it accounted for 26.3 percent of mortalities among women (Audu *et al.,* 2010). These reports sound disturbing and also presents puerperal sepsis as a prevalent source of public health concern particularly in Africa where access to healthcare is suffering severe setbacks due to poverty, weak health institutions, poor application of health policies and a host of other challenges (Balilo et al., 2025). Major clinical manifestations of puerperal sepsis from which complications are to arise are pyrexia/fever, pelvic pain, foul smelling vaginal discharge and delayed reduction of the uterine size after delivery (Van Dillen et al., 2010).

Most postpartum infections take place after hospital discharge and are reportedly caused by endometritis, wound infections, mastitis, urinary tract infections, and septic thrombophlebitis (Say *et al.,* 2014). In most cases, puerperal sepsis arises from infections of the genital tract by pathogens which first of all find their way into the cervix and vagina before gaining access into amniotic fluid from where they eventually invade uterine tissues. Other conditions like maternal anaemia, prolonged labour, increased frequency of vaginal examinations during labour and prolonged rupture of membranes only increase the risk of puerperal sepsis. Implicated bacterial pathogens in the pathogenesis of puerperal sepsis include Peptostretococcus, Clostridia, Pseudomonas, Bactericides fragilis, Escherichia coli, enterococci, Klebsiella spp., Streptococci and staphylococci (Kiponza *et al.,* 2019). The predisposing factors leading to the development of sepsis include home birth in unhygienic conditions, low socioeconomic status, poor nutrition, primiparity, anemia, prolonged rupture of membranes, prolonged labour, multiple vaginal examinations in labour, cesarean sections, obstetrical maneuvers, retained secundines within the uterus and postpartum hemorrhage. Maternal complications include septicemia, endotoxic shock, and peritonitis or abscess formation which may lead to surgery and compromised future fertility (Maharaj, 2007). Puerperal sepsis does not only threaten maternal survival, it also affects child health and is the reason why newborns from mothers with puerperal infection require special attention and should be treated for presumed sepsis (Bennett *et al*., 2015).

Antimicrobial prophylaxis reportedly reduces the incidence of puerperal infection by about 50 percent (David, 2015), however, the current increase in resistance to antibiotics coupled with toxicity concerns regarding the use of most antibiotics remains a sorry of worry (Clark, 2017; Dellicour *et al*., 2017; WHO, 2015; Dondorp *et al.,* 2005). Besides most of the antibiotics are not readily available, even when available, are usually too expensive for the vast majority of patients and women who need them. Therefore the need for a readily available, affordable and effective alternative treatment therefore becomes imperative. The over 80 percent global acceptance and scientifically proven positive antibiotic, antipyretic, antinociceptive and anti-inflammatory properties of most medicinal plants make them promising management alternatives against diseases, including puerperal sepsis (Orieke *et al*., 2019; Ijioma *et al*., 2019), and informs the trial on *Gongronema latifolium, Chromolaena odorata* and *Ocimum gratissinum* in the current study.

Gongronema latifolium (popularly called Utazi in southeast, Nigeria), *Chromolaena odorata* and *Ocimum gratissimum* are common medicinal plants used in Nigeria for the management of divers diseases. The leaves of *Gongronema latifolium* and *Ocimum gratissimum* are usually added to soups, stews, and sauces to create a distinctive taste and provide flavor to the dishes. This study therefore investigated the potencies of these medicinal plants in the management of *Streptococcus agalactiae*-induced puerperal sepsis in rats.

II. Materials And Methods

Collection of plant materials and preparation of extracts

Fresh leaves of *Gongronema Latifolium*, *Chromolaena odarata* and *Ocimum gratissimum* were collected from Amawom-Umudike in Ikwuano Local Government Area of Abia State, Nigeria and were authenticated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State. The leaves were washed with distilled water and allowed to air-dry on a well-ventilated laboratory bench within 21 days. Each dried leaves sample was thereafter pulverized to fine powder using a manual blender and labelled appropriately. The cold maceration technique used by Orieke et al. (2019) was adopted in the extract preparation. Briefly, 300 g of each pulverized sample was macerated in 1500 ml of ethanol (96%) within 48 hours and then filtered, first with a clean handkerchief, then with a filter paper to obtain filtrate which was the extract in ethanol solution. The filtrate so obtained for each sample was dried in a hot oven at 40^{o}C to obtain solid extracts of each plant material. Percentage extract yield for each sample was determined before the extracts were preserved in the refrigerator until needed.

Preliminary phytochemical analysis of the extracts

Phytochemical analysis of *Gongronema latifolium, Chromolaena odarata* and *Ocimum gratissimum* leaf extract was carried bothe qualitatively and quantitatively in accordance with the methods of Trease and Evans (1998) and those outlined in the AOAC (2015) guidelines for phytochemical testing. The phytochemicals tested for flavonoids, alkaloids, tannins, saponins, phenoids, cardiac glycosides, steroids and terpenoids.

Determination of antimicrobial activities of the extracts

The antimicrobial susceptibility profile of the isolates was determined using the agar disc diffusion test (Kirby-Bauer method). Various commercial antibiotics discs were tested against the bacterial isolates while discs of anti-fungal agents were used on the fungal isolates. The bacterial isolates of 0.5 McFarland's turbidity level were inoculated on Muller-Hinton Agar using spread plate method. The antibiotics discs were aseptically placed on the inoculated plates and pressed down using sterile forceps properly. The plates were then incubated at 37^{o}C for 24 – 48 hours. The anti-fungal susceptibility test was carried out on SDA plates. The inoculated of the SDA

plates was also done by the spread plate method. The anti-fungal agents were also aseptically placed on the Agar and pressed down. The plates were incubated at room temperate for 2-3 days. The inhibitory effects of the microbial species by the antibiotics and anti-fungal agents were measured in mm and recorded as Diameter Zone of Inhibition (DZI). The antimicrobial activities of the extracts against the isolates microbial species were also determined using the Agar well diffusion technique. After inoculating the culture media by the spread plate method, 6mm diameter wells were made on the inoculated Agar. Various concentrations of the plant extracts (25%, 50%, 75% and 100% concentrations) were put into the wells separately. The plates were allowed to stand for 20-30 minutes on the table and equally incubated for 24-48 hours (bacteria) and 2-3 days (Fungi). The inhibitory effects were measured in mm and recorded as Diameter Zone of Inhibition (DZI).

Animals

A total of 94 adult female rats (weighing between 110-140 g) were used for the study. Of these rats, 54 were used for lethal dose evaluation of the extracts while 40 were used for the puerperal sepsis study. The rats were housed in well-ventilated aluminum cages in an animal house in the College of Basic Medical Sciences, Abia State University Uturu and allowed access to food and water *ad libitum*. Temperature was maintained at 25 ± 2 ºC, and a 12-h light/dark cycle was provided for the animals throughout the period of the experiment. Humidity levels during the acclimatization and experimental periods ranged from 60- 90%. All experiments were carried out in compliance with acceptable international guidelines for use and care of experimental animals. Ethical approval for the study was obtained from the Abia State University Ethics Committee with approval number: TETFund/IBR/ABSU/ 2021/035).

Determination of lethal dose (LD_{50}) values of the extracts

Lethal dose values of each extract was determined in accordance with Lorke's method with slight modifications as was described by Orieke et al., (2019). Briefly, for each extract, two phases of tests were involved. In the first phase, 9 rats were divided into 3 groups (A, B, C) of 3 animals each and were given oral administrations of the extract at doses 10, 100 and 1000 mg/kg body weight respectively. The animals were thereafter observed for toxicity signs within 6 hours post administration and scored for mortality and general behavior after 24 hours. Due to zero percent mortality recorded across the groups in phase 1, the study proceeded to phase 2 where 9 rats assigned to 3 groups of 3 rats each were also used, but this time treatment doses were 1600, 2900 and 5000 mg/kg body weight respectively. Like in phase 1, the animals were also observed for toxicity signs and mortalities within 6 hours and at the end of 24 hours, then a further 7 days (for phase two). The metrical mean of the maximum dose that produced no mortality and the minimum dose that produced 100% mortality was taken as the mean lethal dose (LD_{50}) of the extract.

Isolation of *Streptococcus agalactiae*

The isolates (*Streptococcus agalactiae*) used to infect animals in this study were obtained from cultured vaginal swab samples of women who attended the Abia State University Hospital in Aba for various delivery complications, especially those suspected to have puerperal sepsis. The vaginal swabs were streaked on Nutrient Agar, McConkey Agar and Sabouround's Dextrose Agar. The swabs were equally streaked on Blood agar base and Tryptic Soy agar enriched with 5% sheep red blood cells. The blood agar was used as enriched media for the isolation of *Streptococcus agalactiae*. The inoculated petri dishes were incubated at 37ºC for 24-48 hours. The *Streptococcus agalactiae* cultured in broth was used to infect the rats by inoculation into the vagina.

Grouping of animals for the puerperal sepsis study

Forty matured female rats already made pregnant by mating with viable males were assigned to 7 groups of 5 rats each and at delivery infected with *Streptococcus agalactiae* by inoculation into the vagina using swab to induce puerperal sepsis. After infection, the various infected groups were treated according to the schedule below:
Group 1: Normal control (uninfected).
Group 2: Infected without treatment (Puerperal control).
Group 3: Infected and treated with *Gongronema latifolium* leaf extract (400 mg/kg body weight).
Group 4: Infected and treated with *Gongronema latifolium* leaf extract (800 mg/kg body weight).
Group 5: Infected and treated with *Chromolaena odarata* leaf extract (400 mg/kg body weight).
Group 6: Infected and treated with *Chromolaena odarata* leaf extract (800 mg/kg body weight).
Group 7: Infected and treated with *Ocimum gratissimum* leaf extract (400 mg/kg body weight).
Group 8: Infected and treated with *Ocimum gratissimum* leaf extract (800 mg/kg body weight).

Treatment was oral and last for 21 days. Body weights of the animals were measured at the beginning and end of treatment period. Animals were sacrificed at the end of treatment after temperature measurments and measurement of pain writhes. Blood collected during sacrifice of the animals into EDTA and plain bottles were used for haematological and serum biochemical analysis respectively.

Measurement of body temperature and pain writhes

Body temperature of each rats was measured by inserting the sensitive point of the clinical thermometer into the vagina of the animal and taking reading within 2 minutes. Pain writhes were measures by observing the animals and noting the number of writhes made by each within 3 hours. The number of writhes made corroborates with the intensity of pain the animals was going through.

Determination of haematological parameters

Haematological parameters including white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), platelets count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and differential white blood cells count were determined using a Mindray automated haematology analyzer (BC-2300, China) in accordance with the protocols outlined by the producer.

Determination of Inflammatory markers

Inflammatory markers including tumor necrosis factor-alpha (TNF-α), interleukin-I beta (1L-Iβ) activity, and interleukin-6 were determined using ELISA techniques and their respective commercial test kits produced by Enzo Life Sciences Inc. NY, USA. Instructions for each test supplied by the test-kit producer were carefully and strictly adhered to arrive at results. The tests awere carried out at the Centre for Molecular Biosciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Histopathological analysis

Method reported by Loha et al. (2019) was used. The uterus of the rats were collected and preserved in 10% formalin. Subsequently, they were washed and embedded in paraffin wax, then sectioned into 5 µm slices before staining with haematoxylin and eosin. An experienced pathologist examined and captured images of the prepared samples using a moticam microscope set at 40x magnification and attached to a computer.

Statistical analysis

The data generated in the study were subjected to statistical analysis, adopting such tools as ANOVA, correlation analysis and any other relevant statistical tools using SPSS version 27.0.

III. Results

Percentage extract yields of the plant materials

Following extraction by cold maceration in ethanol, *Chromolaena odorata* had the highest extract yield (4.13%) and was followed by *Ocimum gratissimum* (3.06%) before *Gongronema latifolium* which had an extract yield of 2.81% (Table 1)

Table 1: Percentage yields of the different plant materials following extraction

Plant sample	Weight of plant material (g)	Weight of extract (g)	Percentage extract yield
Gongronema latifolium	300	8.42	2.807
Chromolaena odarata	300	12.39	4.130
Ocimum gratissimum	300	9.19	3.063

Phytochemical composition of the different plant extracts

Results of phytochemical screening of extracts from the different plants showed the presence of all phytochemicals tested for (Table 2), but in varying quantities. *Chromolaena odarata* had the highest amount of alkaloids and flavonoids when compared with others, *Gongronema latifolium* had the highest amount of terpenoids and steroids, while *Ocimum gratissimum* had the highest amounts of tannins, cardiac glycosides, and saponins with relatively high quantity of alkaloids (Table 3).

Table 2: Qualitative phytochemical composition of the plants

Phytochemicals	*Gongronema latifolium*	*Chromolaena odarata*	*Ocimum gratissimum*
Saponins (mg/100g)	+	++	++
Steroids (mg/100g)	+++	++	+
Flavonoids (mg/100g)	+	++	++
Phenolics (mg/100g)	+	++	+
Terpenoids (mg/100g)	++	+	++
Cardiac glycosides (mg/100g)	+	+	+
Alkaloids (mg/100g)	+	+++	++
Tannins (mg/100g)	+	++	+

Table 3: Quantitative phytochemical composition of the different plant samples

Phytochemicals	*Gongronema latifolium*	*Chromolaena odarata*	*Ocimum gratissimum*
Saponins (mg/100g)	0.90±0.02	2.10±0.02	2.87±0.02
Steroids (mg/100g)	1.21±0.02	0.65±0.02	0.34±0.02
Flavonoids (mg/100g)	2.76±0.02	7.84+00.2	4.01±0.02
Phenolics (mg/100g)	0.89±0.01	1.84±0.01	1.08±0.02
Terpenoids (mg/100g)	3.86±0.02	1.02±0.02	2.84±0.02
Cardiac glycosides (mg/100g)	0.67±0.00	0.84±0.02	1.08±0.02
Alkaloids (mg/100g)	4.35±0.01	8.27±0.01	7.89±0.02
Tannins (mg/100g)	1.00+0.00	1.44±0.00	3.02 ±0.02

Values are presented as mean ± standard deviation (n = 3).

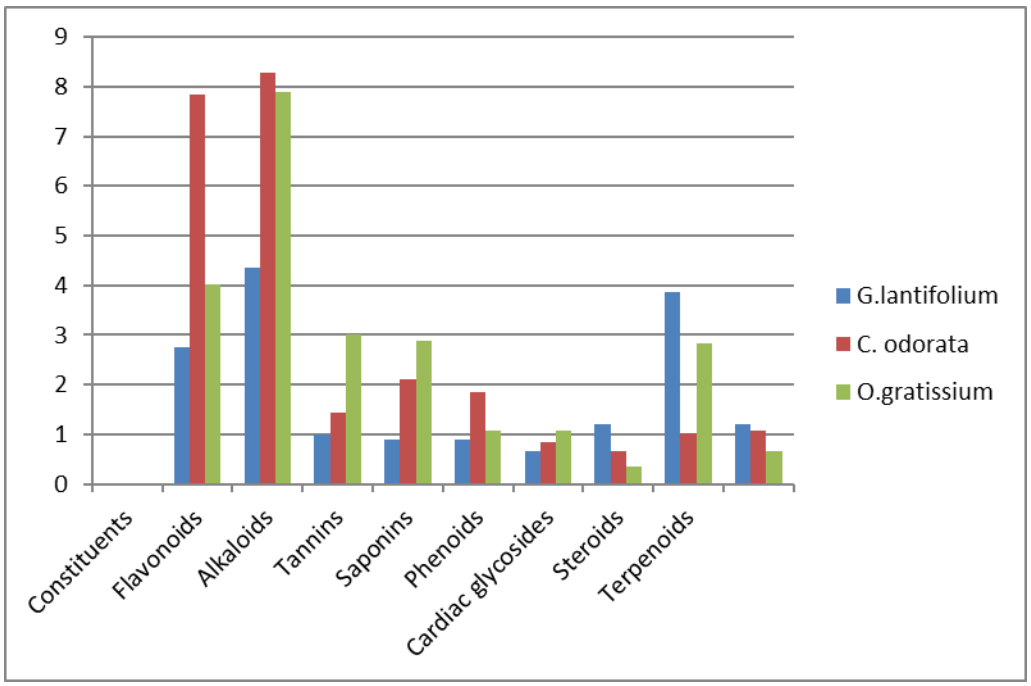


Figure 1: Showing the quantities of the various phytochemicals contained in the plants

Antimicrobial activities of the extracts

Observations of the anti-microbial properties of the three plant extracts showed a similar pattern. The bacterial and Candida species isolated in this work showed resistance to 25% and 50% concentrations of *Gongronema latifolium, Chromolena odorata and Ocimum gratissimum (Tables 4-6)*. However, Results obtained at 75% concentration of each plant extract indicated moderate/intermediate susceptibility of the isolated organisms, but results obtained with 100% of each plant extract gave a diameter zone of inhibition (DZI) which was statistically similar to that of the control antimicrobial agents (Amikacin for bacteria and Amphotericin for *Candida albicans*) (at $p<0.05$). These results are presented in tables 4 – 6.

Tables 4-6: Antimicrobial susceptibility of *Gongronema latifolium* on the isolated microorganisms

Organisms	25% Extract	50% Extract	75% Extract	100% Extract	Control (Amikacin)	
Bacteria *S. agalactiae*	0.4±0.0	7.8±0.2	13.8±0.2	16.2±0.2	18.4±0.2	
S. feacalis	2.6±0.0	9.4±0.3	14.6±0.2	15.2±0.2	19.2±0.2	
N. gonorrhoeae	1.6±0.0	7.8±0.2	13.4±0.2	15.6±0.2	18.9±0.2	
P. aeruginosa	0.8±0.0	8.2±0.2	13.6±0.2	13.8±0.3	16.6±0.3	
Enterococcus species	1.2±0.0	8.8±0.2	15.0±0.2	16.4±0.2	19.6±0.2	
E.coli *Proteus mirabilis*	1.8±0.0 2.4±0.0	8.6±0.2 8.9±0.2	15.4±0.2 14.5±0.2	15.6±0.2 17.0±0.2	19.4±0.2 19.6±0.2	
K. pneumonias Yeast	0.8±0.0	7.8±0.2	12.4±0.2	14.4±0.2	16.6±0.2 Amphotericin B	
Candida albicans	2.8±0.2	.9±0.2	13.6±0.2	15.8±0.2	18.6±0.2	

					Chromolaena odorata	
Organisms	25% Extract	50% Extract	75% Extract	100% Extract	Control (Amikacin)	
Bacteria						
S. agalactiae	0.6±0.2	6.8±0.2	11.9±0.2	15.9±0.2	18.4±0.2	
S. feacalis	2.3±0.2	7.4±0.3	14.0±0.2	15.0±0.2	19.2±0.2	
N. gonorrhoeae	1.4±0.2	7.3±0.2	13.8±0.2	15.0±0.2	18.9±0.2	
P. aeruginosa	0.4±0.0	7.8±0.2	13.8±0.2	14.2±0.3	16.6±0.3	
Enterococcus species	1.8±0.0	8.0±0.2	14.6±0.2	17.2±0.2	19.6±0.2	
E.coli	1.4±0.0	8.0±0.2	16.2±0.2	16.4±0.2	19.4±0.2	
Proteus mirabilis	2.9±0.2	9.4±0.2	12.1±0.2	18±0.2	19.6±0.2	
K. pneumonia	0.6±0.2	7.0±0.2	12.0±0.2	14.0±0.2	16.6±0.2	
Yeast					Amphotericin B	
Candida albicans	2.0±0.2	.4±0.2	13.9±0.2	16.0±02	18.6±0.2	
					O. gratissimum	
Organisms	25% Extract	50% Extract	75% Extract	100% Extract	Control (Amikacin)	
Bacteria						
S. agalactiae	0.2±0.0	6.3±0.2	10.4±0.2	15.4±0.2	18.4±0.2	
S. feacalis	1.6±0.0	6.2±0.2	11.2±0.2	15.6±0.2	19.2±0.2	
N. gonorrhoeae	1.6±0.0	6.6±0.2	11.2±0.2	15.0±0.2	18.9±0.2	
P. aeruginosa	0.6±0.0	6.2±0.2	12.4±0.2	12.0±0.3	16.6±0.3	
Enterococcus species	1.4±0.0	7.2±0.2	13.1±0.2	13.6±0.2	19.6±0.2	
E.coli	1.3±0.0	6.4±0.2	12.2±0.2	15.8±0.2	19.4±0.2	
Proteus mirabilis	1.4±0.0	6.4±0.2	12.2±0.2	18.2.±0.2	19.6±0.2	
Klebsiella pneumonias	0.6±0.0	6.6±0.2	12.4±0.2	14.0±0.2	16.6±0.2	
Yeast					Amphotericin B	
Candida albicans	2.1±0.2	6.4±0.2	12.3±0.2	16.8±0.2	18.6±0.2	

Effects of the extracts on body weight changes in *Streptococcus agalactiae* –induced puerperal septic rats

The disease control rats had significantly lower body weight gain when compared with the normal control group ($p<0.05$). However treatment with the different extracts significantly increased weight gain values in the infected rats, making them significantly higher than the mean value obtained for the disease control group ($p<0.05$). Of the three extract, the highest level of improvement in body weight were observed in the groups treated with *O. gratissimum*, and was followed by *G. latifolium* while *C. odorata* had the least effect (Table 7).

Table 7: Effect of treatment with the extracts on body weight changes

Treatment groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Percentage weight gain
Normal control	136.77±7.58^{a}	144.97±6.57^{c}	8.20±1.40^{d}	6.04±1.30^{d}
Disease (Puerperal sepsis) control	129.93±4.12^{a}	130.67±2.82^{a}	0.73±1.46^{a}	0.59±1.13^{a}
Puerperal sepsis + *G. latifolium* leaf extract (400 mg/kg bw)	131.03±4.05^{a}	133.80±4.46^{a,b}	2.77±1.70^{a,b}	2.11±1.32^{a,b}
Puerperal sepsis + *G. latifolium* leaf extract (800 mg/kg bw)	133.30±1.85^{a}	137.50±1.08^{a,b}	4.20±0.78^{b,c}	3.16±0.62^{b,c}
Puerperal sepsis + *C. odorata* leaf extract (400 mg/kg bw)	130.87±3.61^{a}	132.37±3.00^{a,b}	1.50±0.66^{a}	1.16±0.53^{a,b}
Puerperal sepsis + *C. odorata* leaf extract (800 mg/kg bw)	128.97±4.25^{a}	131.20±4.21^{a}	2.23±0.38^{a,b}	1.73±0.31^{a,b}
Puerperal sepsis + *C. gratissimum* leaf extract (400 mg/kg bw)	135.23±4.35^{a}	139.40±3.06^{b,c}	4.17±1.58^{b,c}	3.11±1.28^{b,c}
Puerperal sepsis + *C. gratissimum* leaf extract (800 mg/kg bw)	131.33±4.30^{a}	137.73±2.36^{a,b}	6.40±2.17^{c,d}	4.91±1.83^{c,d}

Results are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly ($P < 0.05$) different within the column.

Effects of the extracts on body temperature and pain sensations in *Streptococcus agalactiae* –induced puerperal septic rats

The puerperal control rats had significantly higher body temperature when compared with the normal control ($p<0.05$), but treatment with the extracts significantly lowered these elevated temperature values in the puerperal rats with *Ocimum gratissimum* producing the highest body temperature lowering effect and was followed by *Gongronema latifolium* leaf extract (Table 8). Same pattern of results were obtained in the pain writhes tests, where the puerperal treated rats made lower number of pain writhes than the puerperal control rats with *Ocimum gratissimum* and *Gongronema latifolium* leaf extract producing significant lowering effects on writhing reflexes than Chromolaena odorata leaf extract (Table 8).

Table 8: Effect of treatment on body temperature and pain writhes

Treatment group	Body temperature ℃	Pain writhes in 3 hours
Normal control	37.03 ± 0.15^{a}	0.00 ± 0.00^{a}
Disease (Puerperal sepsis) control	38.47 ± 0.25^{d}	10.67 ± 1.53^{e}
Puerperal sepsis + *G. latifolium* leaf extract (400 mg/kg bw)	37.50 ± 0.30^{b}	4.33 ± 0.58^{c}
Puerperal sepsis + *G. latifolium* leaf extract (800 mg/kg bw)	37.43 ± 0.12^{b}	1.67 ± 0.58^{a}
Puerperal sepsis + *C. odorata* leaf extract (400 mg/kg bw)	37.90 ± 0.17^{c}	8.00 ± 1.00^{d}
Puerperal sepsis + *C. odorata* leaf extract (800 mg/kg bw)	37.83 ± 0.15^{c}	7.67 ± 0.58^{d}
Puerperal sepsis + *C. gratissimum* leaf extract (400 mg/kg bw)	37.43 ± 0.15^{b}	2.67 ± 0.58^{b}
Puerperal sepsis + *C. gratissimum* leaf extract (800 mg/kg bw)	$37.27\pm0.12^{a,b}$	2.00 ± 1.00^{b}

Results are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly ($P < 0.05$) different within the column.

Effects of the extracts on haematological parameters in *Streptococcus agalactiae* –induced puerperal septic rat

The puerperal control rats had significantly lower red blood cell count, packed cell volume and haemoglobin concentration when compared with the normal control rats ($p<0.05$), but treatment with the extracts improved the values of these parameters in the puerperal treated rats significantly. Elevated white blood cell count value observed in the puerperal control was also significantly lowered, even though they were still significantly higher than the normal control value, but the significantly reduced platelets count observed following puerperal induction was ameliorated and tilted towards the normal value (Table 9). MCV, MCH and MCHC values were not so significantly altered across the groups, though the puerperal rats had significantly higher MCV values than the normal control (Table 10).

Table 9: Effects of treatment on haematological parameters

Treatment groups	RBC ($x10^6/mm^3$)	PCV (%)	Hb (g/dl)	WBC ($x10^3/mm^3$)	PLT ($x10^3/mm^3$)
Normal control	6.47 ± 0.13^{e}	42.67 ± 0.58^{e}	13.80 ± 0.26^{d}	8.97 ± 0.06^{a}	364.00 ± 7.00^{c}
Disease (Puerperal sepsis) control	5.56 ± 0.12^{a}	38.00 ± 1.00^{a}	12.17 ± 0.15^{a}	12.10 ± 0.38^{d}	287.67 ± 8.51^{a}
Puerperal sepsis + *G. latifolium* leaf extract (400 mg/kg bw)	$6.01\pm0.10^{b,c}$	$40.33\pm0.58^{c,d}$	$13.07\pm0.31^{b,c}$	10.32 ± 0.40^{b}	332.33 ± 18.15^{b}
Puerperal sepsis + *G. latifolium* leaf extract (800 mg/kg bw)	$6.21\pm0.13^{c,d}$	$41.00\pm1.00^{c,d}$	$13.17\pm0.15^{b,c}$	10.04 ± 0.17^{b}	331.00 ± 11.79^{b}
Puerperal sepsis + *C. odorata* leaf extract (400 mg/kg bw)	5.61 ± 0.15^{a}	$38.67\pm0.58^{a,b}$	12.77 ± 0.25^{b}	11.28 ± 0.35^{c}	355.00 ± 8.89^{c}
Puerperal sepsis + *C. odorata* leaf extract (800 mg/kg bw)	5.85 ± 0.04^{b}	$39.67\pm0.58^{b,c}$	$12.93\pm0.31^{b,c}$	11.13 ± 0.27^{c}	352.33 ± 9.87^{c}
Puerperal sepsis + *C. gratissimum* leaf extract (400 mg/kg bw)	5.93 ± 0.20^{b}	$39.67\pm1.16^{b,c}$	$13.00\pm0.20^{b,c}$	10.12 ± 0.15^{b}	298.67 ± 6.66^{a}
Puerperal sepsis + *C. gratissimum* leaf extract (800 mg/kg bw)	$6.29\pm0.06^{d,e}$	$41.67\pm0.58^{d,e}$	13.33 ± 0.21^{c}	9.95 ± 0.19^{b}	321.00 ± 10.15^{b}

Results are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly ($P < 0.05$) different within the column.

Table 10: Effects of treatment on more haematological parameters

Treatment groups	MCV (fl)	MCH (pg)	MCHC (g/dl)
Normal control	65.95 ± 0.53^{a}	$21.33\pm0.05^{a,b}$	$32.34\pm0.21^{a,b,c}$
Disease (Puerperal sepsis) control	$68.38\pm0.55^{d,e}$	21.90 ± 0.25^{c}	32.03 ± 0.45^{a}

Puerperal sepsis + *G. latifolium* leaf extract (400 mg/kg bw)	67.15±0.37^{b,c}	21.75±0.14^{c}	32.39±0.35^{a,b,c}
Puerperal sepsis + *G. latifolium* leaf extract (800 mg/kg bw)	66.02±0.30^{a,b}	21.21±0.23^{a}	32.12±0.42^{a,b}
Puerperal sepsis + *C. odorata* leaf extract (400 mg/kg bw)	68.90±0.86^{e}	22.75±0.22^{b,c}	33.02±0.28^{c}
Puerperal sepsis + *C. odorata* leaf extract (800 mg/kg bw)	67.77±0.80^{c,d}	22.09±0.39^{c}	32.60±0.36^{a,b,c}
Puerperal sepsis + *C. gratissimum* leaf extract (400 mg/kg bw)	66.94±0.82^{a,b,c}	21.94±0.40^{c}	32.78±0.57^{b,c}
Puerperal sepsis + *C. gratissimum* leaf extract (800 mg/kg bw)	66.24±0.49^{a,b}	21.20±0.23^{a}	32.00±0.13^{a}

Results are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly (P < 0.05) different within the column.

Effect of the extracts on some inflammatory cytokines levels in *Streptococcus agalactiae* –induced puerperal septic rat

Serum concentrations of inflammatory cytokines including interleukin 1beta, interleukin-6 and tumor necrosis factor alpha were significantly higher in the induced puerperal rats when compared with the normal/un-induced control group, but treatment with all extracts significantly lowered the levels of these markers with *Ocimum gratissimum* producing a better lowering effect than other extracts (Table 11).

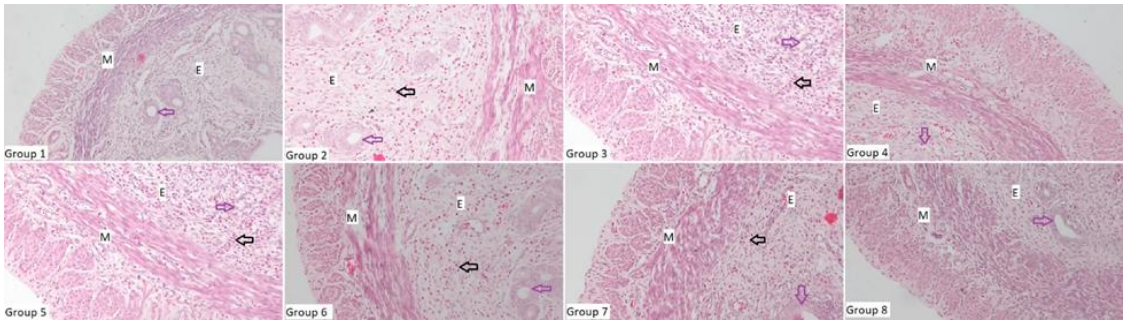
Table 11: Effect of treatment on inflammatory markers

Treatments	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)
Normal control	5.12±0.38^{a}	0.37±0.05^{a}	18.99±1.92^{a}
Disease (Infected) control	14.68±0.67^{f}	1.99±0.13^{d}	42.30±2.81^{e}
Infected + *G. latifolium* leaf extract (400 mg/kg bw)	9.49±1.00^{c}	0.78±0.22^{b}	29.52±33.90^{c}
Infected + *G. latifolium* leaf extract (800 mg/kg bw)	7.90±0.42^{b}	0.77±0.06^{b}	24.01±1.90^{b}
Infected + *C. odorata* leaf extract (400 mg/kg bw)	11.83±0.66^{e}	1.70±0.21^{c}	33.97±1.52^{d}
Infected + *C. odorata* leaf extract (800 mg/kg bw)	10.81±0.41^{d}	1.59±0.04^{c}	33.61±3.10^{c,d}
Infected + *C. gratissimum* leaf extract (400 mg/kg bw)	10.22±0.15^{c,d}	0.93±0.09^{b}	24.11±1.92^{b}
Infected + *C. gratissimum* leaf extract (800 mg/kg bw)	9.49±0.20^{c}	0.78±0.05^{b}	23.41±1.27^{b}

Results are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly (P < 0.05) different within the column.

Observed uterine histopathological changes in *Streptococcus agalactiae*–induced puerperal septic rats treated with the extracts

Severe degenerations and increased infiltration of inflammatory cells were observed in the untreated infected rats, while rats treated with extracts had these pathological states significantly ameliorated with 800 mg/kg of *Ocimum gratissimum* restoring the uterine histological architecture to almost normal state (Plates presented as groups 1-8).



Photomicrograph of the uterus of Group 1 – 8, showing the endometrium (E), endometrial glands (purple arrow), uterine muscle (M). There is severe infiltration of inflammatory cells (black arrow) in the endometrium of Group 2; moderate infiltration in Groups 5 and 6, and mild infiltration in Groups 3 and 7. Groups 1, 4 and 8 have normal morphology with no cellular infiltration. H&E. X100 magnification.

IV. Discussion

In this study, the potencies of Gongronema latifolium, Chromolaena odorata and Ocimumm gratissimum leaf extracts in the management of Streptococcus agalactiae-induced puerperal sepsis were investigated in rats with results showing positive outcomesfor Gongronema latifolium and Ocinum grstissimum leaf extracts. Results of their phytochemical analysis reported in study agrees wit the findingsof study of Ajobi *et al*.,(2012) and Eleyinmi, (2007) for Gongronema latifolium. Chromolaena odorata and Ocimum gratissimum leaf

extracts are non-toxic to the systemfollowing acute administration, having produced no mortality, even at 5000mg/kg body weight oral dose. This conclusion is consistent with the deductions of Ijioma *et al*., (2021) and Lappa *et al*., (2021) in their separate evaluations of acute toxicity activities of plant extracts.

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