GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Evaluation of anti-microbial and anti-inflammatory properties of ethanol extract of *Allium sativum linn*

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GSC Biological and Pharmaceutical Sciences, 2022, 19(02), 044–056

Publication history: Received on 07 March 2022; revised on 17 April 2022; accepted on 17 April 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.19.2.0143

Abstract

This study evaluated the anti-microbial and anti-inflammatory properties of ethanol extract of *Allium sativum linn.* The ethanol extract yielded two distinct layers which differed in phytochemical composition, anti-microbial and anti-inflammatory properties. In the base filtrate (BF), tannins, carbohydrates, alkaloids, glycosides, reducing sugars, flavonoids, saponins, acidic compounds and resins were found present. The methanol fraction of the base filtrate was however found to have resins and tannins. The acute toxicity (oral) in this study was 3,750 mg/kg. Anti-microbial activity was tested using clinical isolates of *Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, Salmonella typhi,* and *Klebsiella pneumonia,* with Penicillin-G, Erythromycin, Gentamicin, Ciprofloxacin and Ceftriazone as standard drugs. The upper filtrate and base filtrate of the extract were found to exhibit a statistically significant and dose-dependent inhibitory effect on all test micro-organisms when compared with control. The result of the systemic anti-inflammatory study showed that the extract had a statistically significant, dose and time dependent systemic anti-inflammatory effect when compared with the negative and positive controls. The topical anti-inflammatory study showed a statistically significant, dose and time dependent topical anti-inflammatory effect at all tested doses.

In conclusion, this study was able to establish that extract of *Allium sativum* had a statistically significant ($p < 0.05$) anti-microbial and anti-inflammatory effects, which is comparable to standard anti-microbial and anti-inflammatory drugs. This pharmacological effect may be due to the abundant presences of phytochemical constituents in the ethanol extract of *Allium sativum L.* This goes a long way to support its extensive use as an anti-microbial and anti-inflammatory agent in ethno medicine.

Keywords: *Allium Sativum* L; Anti-Microbial; Anti-Inflammatory; Activity

1. Introduction

Medicinal plants have been the main stay of traditional medicine amongst rural dwellers worldwide since antiquity to date [1]. A single plant may be used for the treatment of various diseases depending on the community [2,3]. Different plant parts and components (roots, leaves, stems barks, flowers, essential oil, or their combination) have been employed in the treatment of infectious disease [4]. Medicinal plants discovered by traditional societies are proving to be important sources of potential therapeutic drugs [5,6], while treatment of aliments with herbal drugs as first line or adjuvant seems to be beneficial and is gaining popularity especially in developing countries [7]. Drugs from plant origin are used in developing countries for the treatment of many diseases. Such therapies may be helpful in controlling the symptoms of acute and chronic inflammatory diseases [8], WHO encourages the inclusion of herbal medicines of proven safety and efficacy in the health programs of developing countries [7].

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Allium sativum commonly known as garlic is a species in the onion genus, *Alliaceae.* Its close relatives include the onion, shallot, leek, chive and rakkyo [9]. The genus *Allium* has about 1,250 species, making it one of the largest plant genera in the world [10,11]. With a history of human use of over 7,000 years, garlic is native to central Asia, but nowadays it is cultivated all over the world [12,13]. It was known even at the time of the pharaohs and has been used for both culinary and medicinal purposes [14].

Allium sativum has been reported to have a wide range of medicinal properties like anti-microbial, antiviral, antifungal, antihelminthic, anti-inflammatory, antidote, anti-carcinogenic, anti-mutagenic, hepato-protective, anti-genotoxic immune-modulation, and many others [15,16,17].

With numerous publications on the chemistry and biological effects of garlic, one gets the impression that garlic is a thoroughly investigated medicinal plant. However, studies addressing the immune-modulatory effects of garlic reveal conflicting data as to pro- or anti-inflammatory, responses depending on the particular experimental set-up and the garlic preparation used (i.e. garlic extract versus chemically pure garlic compounds) [18].

An integrated host inflammatory response is essential in maintaining health and fighting against diseases, but pathological inflammation is linked to many ailments. Non-steroidal anti-inflammatory drugs (NSAIDs), used to treat inflammation and pain, are among the most commonly prescribed drugs worldwide to treat both acute and chronic inflammations, but its use is hampered by NSAIDs-related adverse effect, mainly gastrointestinal, a fact that justifies the search for new anti-inflammatory substances [19].

Infection is an invasion of the body by infectious organisms such as viruses, bacteria, fungi or parasites [20]. The concept of an infectious agent was established by Robert Koch in the late 19th century. This process which contaminates the body can cause injury by harming various tissues of the body. When the body is injured, it calls upon its defenses to protect it by a process called inflammation. The human host relies on innate and adaptive immune and inflammatory responses to control the normal flora and respond to pathogens [21]. Inflammation thus jump-starts the healing process. If it did not occur, infection could run rampant, causing more damage and destruction inside the body. This could lead to further injury or permanent damage to tissues. Any agent that offers treatment of both infection and inflammation will help maximize the body's chance for recovery [22,23,24]. Thus the need to find out the anti--inflammatory and anti-microbial effect of *Allium sativum* if any, and the active constituents which brings about these effects cannot be over stated.

Several recent studies has shown that sulphur containing plants such as *Allium sativum* can effectively interfere with the transcription of various inflammatory cytokines, such as interleukin-1, interleukin-2, interleukin- 6, interleukin-beta and tumour necrosis factor-alpha, as well as genes encoding cycloxygenase-2 (Cox-2) and intrinsic Nitric oxide synthetase (iNOS), and as a result, inhibit the signal pathway leading to activation of the inflammatory process [25]. Among these works on *Allium sativum,* none has been done with our local garlic to confirm or disprove these statements; hence these studies.

2. Material and methods

2.1. Collection and Identification of Plant Material

About 3kg of fresh garlic was bought from Ngwo Ibeagwa-aka market, Nsukka, Nsukka LGA, Enugu State, Nigeria and was identified and authenticated by Mr. A. Ozioko, a Taxonomist, of the Bio-research Development and Conservation Programme (BDCP) Centre, Nsukka, Enugu state, Nigeria.

2.2. Laboratory Animals

Male and female Swiss albino mice weighing 22-28 grammes were used for acute toxicity study while Wistar albino rats weighing 180-200 grammes were used for the pharmacological studies. They were obtained from the Department of Pharmacology and Toxicology, Animal house, University of Nigeria, Nsukka. They were fed with grower mesh of vital feeds, Jos, Nigeria, purchased from a local supplier and housed under natural conditions of temperature and illumination. The animals were allowed free access to feed and drinking water *ad libitum* for a period of 2 weeks prior to the start of the experiment.

2.3. Extraction of Plant Material (Cold Maceration)

2.3.1. Extraction of ethanol sample of Allium sativum

The fresh three (3) kilogram of *Allium sativum* was washed, air-dried and pulverized with a blender. The fine pulverized paste was placed in two 5 litres maceration flasks; having 1.5 kg in each flask with 5 litres of ethanol. A frequently shaking of the flask started immediately after the mixture and lasted for 72 hours at room temperature. At the end of this time, it was filtered with a porcelain cloth and then a filter paper for fine filtration. The filtrate was concentrated and evaporated with a rotary evaporator until constant weight was achieved.

2.3.2. Extraction of n-Hexane, Ethyl Acetate and Methanol Fractions

Exactly 50grammes of the ethanol extract was thoroughly mixed with l00 grammes of silica gel and was partitioned into n-hexane, ethyl acetate and methanol in a glass column (according to their polarity differences). The resulting fractions were evaporated at less than 40°c in a rotary evaporator.

2.3.3. Phytochemical Analysis

Phytochemical analysis was carried out on the extract at the Pharmacology and Toxicology Laboratory of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, using standard procedures as described by[26,27].

2.3.4. Determination of acute toxicity (ld50)

Acute toxicity of the extract was performed using a modified [28] method. Male and female Swiss albino mice weighing 22-28(grammes) and housed under standard conditions of temperature and humidity and fed standard meal and clean water were used. The vehicle throughout the duration of the study was 3% Tween 80 solution. Two animals were used at the higher doses.

2.3.5. Microorganisms used

The microorganisms that were used for this research were clinical isolates of two Gram positive and three Gram negative bacteria. They include *Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, Salmonella typhi,* and *Klebsiella pneumonia.*

Sterile swab sticks of the samples were transferred from hospital patients and were characterized macroscopically up to specie level according to cultural characteristics (shape, edge, texture, odour, among others) and cell characteristics (gram staining and catalase test) using standard procedure [29] at the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The purity and identification of each of the bacteria isolates was also confirmed using the standard procedures of biochemical tests [29].

The test bacteria were standardized using 0.5 Mcfarland standard turbid equivalents [29].

2.3.6. Standard Anti-microbial drugs used

Pure drug samples of Penicillin-G, Erythromycin, Gentamicin, Ciprofloxacin and Ceftriazone were obtained from Juhel Pharmaceutical Limited, Enugu. These were used for the comparative minimum inhibitory concentration (MIC) studies.

2.4. Experimental Method

A 2.8gramme of hydrated Nutrient agar was suspended in 100 ml of distilled water and was allowed to soak. The suspension was melted by boiling in a water bath. A 20 ml volume of the molten nutrient agar was dispensed in bujio bottles each and sterilized at 121 °C for 15minutes. The sterile media was kept at 40 °c until used.

A 0.1ml of the standardized test microorganism was seeded into a sterile petri dish with a 20 ml of sterile molten agar kept at 40°c, and the agar plates were allowed to gel. The nutrient agar was divided into five equal segments with a permanent marker to correspond with the concentrations. Holes of 6 mm diameter were bored in each segment, and two drops of drug suspension and extract was placed into the holes according to their corresponding concentrations.

The plates were incubated at 37 °C for 24 hours. After 24 hours incubation the plates were observed and the inhibition zone diameter (IZD) were measured and recorded.

2.4.1. Standard Anti-inflammatory Drug

Pure drug sample of Piroxicam was obtained from Juhel Pharmaceutical Limited Enugu. This was used as positive control for the systemic anti-inflammatory study.

2.4.2. Systemic acute inflammation

The rat paw oedema method of [30] was used. The animals were weighed using an electronic weighing scale with weight range of 162-181 grammes and were grouped into five (5), having five (5) animals per group. Doses were administered based on the body weight of each animal using the formulae.

$$\text{Dose (mg/kg)} = \text{dosage} \times \text{weight}$$

1000 (converting weight in gramme to kg)

The experiment was done in two phases. In phase one, the ethanol extract 1, extract II and the methanol fractions were administered at dosages of 100, 500, and 900mg/kg orally. In phase two, the ethanol extract 1, extract II and the methanol fractions were administered at dosages of 1000, 1500, and 2000 mg/kg orally. The control animals received 5 ml/kg of the vehicle (3% v/v Tween 80), as negative control while positive control were given 5 mg/kg of Piroxicam. The doses were selected based on arithmetic progression and it was ensured that the doses did not exceed the LD 50 which was 3,750 mg/kg.

Thirty minutes after extract administration, inflammation was induced by subplantar injection of 0.1 ml of fresh egg albumin in the hind paw [31]. The volume of the paw was measured by water displacement at 0 min, 30min, 1, 2, 3, and 4 hours after induction of inflammation. The level of inhibition of oedema was calculated for each extract/fraction using the relation [31].

$$\text{Inhibition (\%)} = 100\ (1- (a-x)/ (b-y)$$

Where A = mean paw volume of treated animals after egg albumin injection; x = mean paw volume of treated animals before egg albumin injection; b = mean paw volume of control animals after the egg albumin injection y = mean paw volume of control animals before egg albumin injection.

2.4.3. Topical acute inflammation

The effect of extract on topical acute oedema was assessed using xylene-induced ear oedema in mice. Swiss albino mice weighing 22-28 grammes, received topical application of crude extract I (first layer), extract II (lower layer) and methanol fractions of 50, 100 and 200 mg respectively on the anterior surface of the right ear, while xylene was instantly applied on the posterior surface of the same ear. Control animal received an equivalent volume of the vehicle (3% v/v Tween 80). The left ear was left untreated. Fifteen minutes after xylene application, the mice were sacrificed and both ear lobes cut at the base of the ear. Using a 6 mm diameter cork bore, equal portions of both ears were cut and weighed. The difference in the weights from the right treated and left untreated ears shall be calculated and used as a measure of oedema [32].

The level of inhibition (%) of oedema was calculated using the relation:

$$\text{Inhibition (\%)} = 100(1- (Et/Ec)$$

Where: Et = average oedema of the treated group.
Ec = average oedema of the control group.

2.5. Data Analysis

Results of the experiment was subjected to a one way Analysis of Variance (ANOVA) using statistical package for social sciences (SPSS) version 16 and presented as Mean ± Standard error of mean. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Determination of Percentage Yield

Total weight of pulverized garlic was 2500 gm (2.5 kg). Two distinct layers of ethanolic extract of *Allium sativum* were obtained from the two flasks as shown in figure 1.

Total yield of the upper filtrate (UF) of extract of *Allium sativum* was 628.52 grams (25.14%) while the total yield from the base filtrate was 720.15 (28.81%).

3.2. Percentage yield of N-Hexane, Ethyl Acetate and Methanolic Fractions

The resulting fractions were evaporated at less than 40°C in a rotary evaporator. Methanol which was more polar gave a yield of 6.2grams (12.4%), while n-hexane, chloroform, ethyl acetate and acetone gave no yield.

3.3. Result of acute toxicity

LD_{50} determination was done using a modified Lorke's method. The LD_{50} was done orally and the result was gotten from the geometrical mean of the maximum therapeutic dose and the least lethal dose.

The LD_{50} was 3,750 mg/kg.

A scale proposed by [28] roughly classifies substances according to their LD50 as follows: Very toxic, LD50 < l.0mg/kg; Toxic, LD_{50} up to l0 mg/kg; less toxic, LD_{50} up to l00 mg/kg; slightly toxic, up to l000mg/kg, while value of 5000 mg/kg and above are practically non toxic

In this study, 96% ethanol extract of *Allium sativum* yielded two distinct layers with differences in phytochemical constituents, anti-microbial and anti-inflammatory properties. The upper filtrate (UF) of the extract had an abundance (+++) of glycosides, resins and reducing sugars while there was moderate (++) presence of alkaloids, flavonoids, tannins, saponins, acidic compounds and carbohydrates. The base filtrate (BF) had abundant (+++) presence of tannins, and carbohydrates, and moderate (++) presence of alkaloids, glycosides, reducing sugars, flavonoids, saponins and acidic compounds, with trace presence of resins. Fats and oils, steroids, proteins and terpenoids were absent in the ethanol extract. The methanol fraction however gave only moderate presence of resin and tannins.

Table 1 Phytochemical constituents of extract and fractions

Constituent	EE (UF- BP) I	EE (BF) II	MEOH Fr
Alkaloids	++	++	-
Glycosides	+++	++	-
Reducing sugar	+++	++	-
Tannins	++	+++	++
Resins	+++	+	++
Steroids	-	-	-
Terpenoids	-	-	-
Protein	-	-	-
Fats & Oil	-	-	-
Flavonoids	++	++	-
Saponnins	++	++	-
Acidic compounds	++	++	+
Carbohydrate	++	+++	-

EE (UF-BP) I = Ethanol extract upper filtrate (Brown-paste) EE (BF) II=Ethanol extract base filtrate MEOH Fr = Methanol fraction; +++ = Conspicuously Present; ++ = moderately present; + = Present in small quantity;- = absent

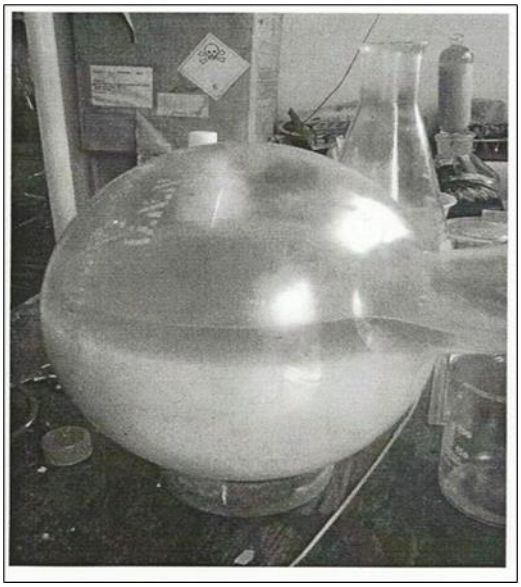

Figure 1 The upper and lower filtrate of ethanol extract of *Allium sativum linn*

Table 2 Statistical analysis of MIC of drugs/extract at different concentrations

Conc. Std. Drugs	***Staph, aureus***	***Strep, pneumonia***	***E. coli***	***S. typhi***	***k. pneumonia***
1.25 µg Penicillin	31.67 ±.33**	18.001.06**	.001.00**	.001.00**	.001.00**
Cipro.	26.00 ±.58**	15.001.58**	12.3311.20**	20.00 + 1.15**	34.67 1 4.06
Genta.	22.67 ±.89**	16.001.58**	11.671.89**	15.00 + 1.15**	15.33 + 1.76**
Ceftri.	29.00± .58**	27.0012.08**	19.0011.15**	16.001.58**	26.001.58
Erythro.	34.0011.15**	15.001.58**	.001.00**	20.00 + 1.15**	11.331.89**
Garlic Ext	31.331.67**	14.001.58**	.00 1.00**	.001.00**	27.3313.84
3% DMSO	44.6711.76	34.0014.04	34.0014.04	34.00 1 4.04	34.0014.04
2.5 µg Penicillin	28.001.58**	19.331.88**	.001.00**	.001.00**	.001.00**
Cipro.	22.001.58**	10.67 1.88**	10.671.88**	16.00 + 1.15**	28.3312.03
Genta.	19.001.58**	11.671.33**	8.001.58**	12.3311.76**	13.00+1.15**
Ceftri.	23.67 11.45**	12.331.88**	16.0011.15**	13.00 + 1.15**	21.331.67**
Erythro.	27.001.58**	12.0011.15**	.001.00**	17.00 + 1.15**	9.00 + 1.15**
Garlic Ext.	25.3311.20**	10.3311.76**	.001.00**	.001.00**	20.0011.15**
3% DMSO	45.3312.33	30.6712.40	- 30.67 1 2.40	30.6712.40	30.67 1 2.40
5 µg Penicillin	22.67 1.88**	14.671.88**	.001.00**	.00 + .00**	.001.00**
Cipro.	17.001.00**	8.0011.15**	8.001.57**	.0011.15**	22.6711.45**
Genta.	16.001.58**	11.331.67**	.001.00**	10.00 + 1.15**	10.00+1.15**
Ceftri.	20.0011.15**	19.001.58**	12.0011.15**	10.001.58**	16.671.88**
Erythro.	23.331 1.45**	8.671.33**	.001.00**	14.001.58**	.001.00**
Garlic Ext	21.331.88**	8.3311.76**	.001.00**	.00 1.00**	15.00 + 1.15**

3% DMSO	47.6713.18	31.3311.76	31.33 + 1.76	31.3311.76	31.33 + 1.76
10 µg Penicillin	18.001.58**	12.0011.15**	.001.00**	5.001.00**	.00 + .00**
Ciproflo.	14.001.58**	.67 1.67**	.001.00**	8.001.58**	17.001.15**
Genta.	14.00 + 1.15**	10.0011.15**	.001.00**	.0011.15**	7.001.15**
Ceftri.	16.001.58**	15.0011.15**	8.0011.15**	7.331.88**	13.331.88**
Erythro.	17.671.88**	9.0011.15**	.001.00**	10.001.58**	.001.00**
Garlic Ext.	16.001.58**	7.0011.15**	.001.00**	5.001.00**	9.0011.15**
3% DMSO	46.0014.16	30.6711.33	30.67 11.33	30.67 + 1.33	30.6711.33**
20 µg Penicillin	14.001.58**	9.0011.15**	.001.00**	.001.00**	.001.00**
Ciproflo.	9.001.58**	.001.00**	.001.00**	.00 + .00**	10.331.88**
Genta.	9.0011.15**	8.0011.15**	.001.00**	.00 + .00**	.00 1.00**
Ceftri.	10.001.58**	11.0011.15**	.001.00**	.00 + .00**	8.331.88**
Erythro.	12.671.88**	.001.00**	.00 + .00**	6.33 + 1.45**	.001.00**
Garlic Ext.	13.331.33**	.001.00**	.001.00**	.001.00**	.001.00**
3% DMSO	45.3314.37	40.6712.33	40.67 1 2.33	40.67 + 2.33	40.6712.33

** = shows concentration at which statistically significant (p< 0.05) inhibitory effect is obtained.

3.4. Statistical analysis of MIC

Evaluation of the anti-microbial properties of ethanol extract of *Allium sativum* showed a statistically significant ($p < 0.05$) and dose-dependent inhibitory effect on all test microorganisms when compared with control, and better than Penicillin and Erythromycin except for *Klebsiella pneumonia.* Ethanol extract of *Allium sativum* had a statistically significant ($p < 0.05$) inhibitory effect on all test micro-organisms and at all concentrations tested except for *Klebsiella pneumonia* where inhibition occurred at higher concentration.

There was complete inhibition of growth of *Escherichia coli* and *Salmonella typhi* at concentrations of 1.25 µg to 20 µg. There was also complete inhibition of all visible growth of *Streptococcus pneumonia, Eschericia coli, Salmonella typhi* and *Klebsiella pneumonia* at higher doses. There was incomplete inhibition of *Staphylococcus aureus* at all test concentrations.

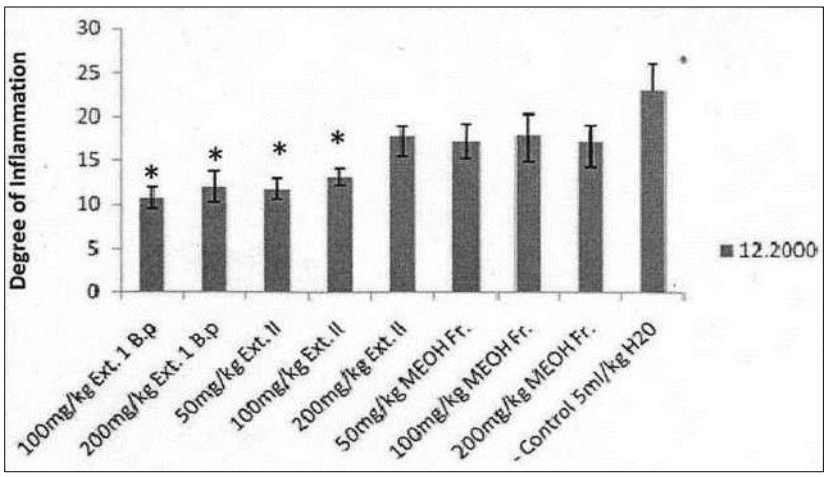

Figure 2 Topical anti-inflammatory effect of garlic extracts/fraction on Xylene-induced mice ear oedema

The study of the topical anti-inflammatory effect of ethanol extract of *Allium sativum* on xylene- induced ear oedema in mice showed that the effect of extract I (UF) of 100 and 200 mg and extract II (BF) of 50 and l00 mg applied on the ear oedema of mice had a statistically significant effect when compared with negative control, distilled water.

Systemic anti-inflammatory effect of garlic extracts/fraction on Egg albumin-induced Rat Hind Paw oedema (1 and 11)

Table 3 Effect of extract on egg albumin-induced Rat Hind Paw oedema (I)

Ext./Drug	Dose(mg/kg Oh		30min	Lh	2h	3h	4h
Ext.l	100	.50 ± .04	1.40 ± .05	1.48 ±.07	1.40 ±.05	1.28 ±.04	1.16 ± .05
"	500	.54 ± .04	1.48 ± .06	1.54 ± .04	1.46 ± .06	1.34 ± .07	1.20 ± .05
	900	.46 ± .02	1.42 ± .04	1.56 ± .04	1.40 ± .03	1.24 ± .04	1.06 ± .04
Ext. II.	100	.50 ± .03	1.46 ± .04	1.56 ±.04	1.50 ± .03	1.46 ±.04	1.34 ±.04
	500	.55 ± .05	1.52 ±.067	1.58 ± .049	1.52 ± .07	1.52 ±.07	1.38 ± .07
	900	.52 ± .06	1.48 ± .08	1.54 ±.07	1.38 ±.09	1.32 ±.07	1.14 ± .09
MEOH fr. 100		.54 ± .06	1.52 ± .05	1.66 ± .04	1.56 ± .04	1.46 ± .05	1.28 ± .07
"	500	.52 ± .04	1.52 ± .04	1.64 ± .02	1.50 ± .03	1.46 ± .05	1.24 ± .02
"	900	.44 ± .02	1.40+ .03	1.52 ± .04	1.44 ± .02	1.40 ± .03	1.38 ± .16
D. H_20	5 ml/kg	.54 ± .06	1.50 ± .04	1.62 ± .02	1.54 ± .02	1.40 ± .04	1.24 ± .05

The result showed that the extract had no statistically significant anti-inflammatory effect when compared with the negative control group (5 ml/kg distilled water)

Table 4 Effect of extract on egg albumin-induced Rat Hind Paw oedema (II)

Ext./Drug	Dose(mg/kg Oh		30min	Lh	2h	3h	4h
Ext.l	1000	.50 ±.04	1.66 ± .05	1.46 ±.05	1.46 ±.05**	1.30 ±.03	1.18 ±.04**
(UF)	1500	.54 ± .04	1.50 ±.03	1.58 ±.04**	1.36 ±.04**	1.16 ± .05**	.92 ± .04**
	2000	.46 ± .02	1.42 ±.04	1.48 ± .04**	1.28 ±.04**	1.08 ±.04**	.78 ±04**
Ext. II.	1000	.50 ± .03	1.48 ±.05	1.48 ±.06	1.56 ± .04	1.38 ± .05	1.16 ± .02**
(BF)	1500	.55 ± .05	1.50 ±.04	1.50 ± .04	1.58 ±.05	1.26 ± .02*	1.10 ± .03**
	2000	.52 ± .06	1.58 ± .06	1.58 ±.05	1.54 ±.07	1.10 ± .04	.94 ± .02**
MEOH fr 1000 .54 ±.06 1			1.52 ± .02	1.52 ± .04	1.66 ±.04	1.56 ±.04	1.36 ±.05
"	1500	.52 ± .04	1.46 ±.04	1.50 ±.04	1.66 ±.04	1.38 ± .04	1.18 ± .04
	2000	.44 ± .024	1.48 ± .04	1.48 ±.04	1.52 ± .04	1.36 ±.05	1.06 ± .04*
Piroxicam 5mg/kg .55 ± .03			1.44 ±.02	1.34 ± .02**	1.24 ± .02**	1.02 ± .02**	.84 ± .02**
D. H_20 5ml/kg		.54 ± .058	1.54 ± .07	1.54 ±.07	1.70 ±.04	1.48 ±.04	1.42 ± .04

** = shows level at which systemic anti-inflammatory effect became significant

3.5. Results of Effect of Extract on Egg Albumin-Induced Rat Hind Paw Oedema (I and Ii)

The study was carried out in two stages. In the first stage (table 3.), doses of l00 mg/kg, 500 mg/kg and 900 mg/kg of the extract were administered to the study animals and there was no statistically significant effect when compared with the negative control group (5 ml/kg distilled water).

In the second stage (table 4), doses of l000 mg/kg, 1500 mg/kg and 2000 mg/kg were used. The result showed that at 2nd, 3rd and 4th hours for the upper filtrate of the extract (UF) there was a statistically significant effect at 1000, 1500 and 2000 mg/kg respectively. At the fourth hour, the base extract of *Allium sativium* at all the doses was statistically significant while at 2000 mg/kg, the Methanol fraction was statistically significant.

At one hour, the upper filtrate of the extract had same statistical significance ($p< 0.05$) for both 1500 mg/kg and 2000 mg/kg respectively while at the 2nd hour the 1500 and 2000 mg/kg had statistical significance of $p< 0.05$ against Piroxicam that had $p< 0.01$.

At the 3rd and 4th hour the upper filtrate of the extract had same level of significance with Piroxicam with $p < 0.05$ for the 1500 mg/kg and 2000 mg/kg doses.

4. Discussions

Allium sativum has been investigated extensively for health benefits, over the last decade alone. It is considered one of the best disease preventive herbal plants based on its potent and varied effects [33]. However recent studies cast doubt on the health benefits of *Allium sativum*, including its anti-microbial and anti-inflammatory effects[34,18.]

Much of the protective and pharmacological effects of fruits and vegetables have been attributed to phytochemicals which are non-nutrient plant compounds such as carotenoids, flavonoids, saponins, phenolic compounds, and many others [35]. While there are still many others that have not been identified [36]. In this study, ethanol extract of *Allium sativum* was shown to contain various phytochemicals which could contribute to its pharmacological effect.

The phytochemical analysis in this study however differs from the work of [37] that gave the phytochemical constituents of *Allium sativum* extract as saponins, steroids, tannins, carbohydrates, and cardiac glycosides whereas alkaloids, flavonoids, anthraquinones, cardenolides and cyanogenic glycosides were found absent .[38] In their study found an abundance of tannins and moderate presence of alkaloids, flavonoids and saponins in ethanol extract of *Allium sativum*. However, the work of [39], posited that ethanol extract of *Allium sativum* had a low presence of phyotochemicals. They also showed that 70% ethanol and methanol gave less yield and less active constituents than 98% ethanol and methanol. This study on the other hand, was able to show an abundance of phytochemicals in 96% ethanol extract of *Allium sativum*. This is possible because concentration of plant constituents of the same plant organ can vary from one geographical location to another depending on the age of the plant, difference in geographical factors, the nutrient concentrations of the soil, extraction method as well as method used for the study 1.

Tannins cause inhibitions in the cell wall synthesis by forming irreversible complexes with prolene rich proteins [40]. They have also been reported to exhibit antiviral, antibacterial, anti-tumour activities while enhances the cell proliferation, tissue regeneration and wound healing processes [41, 42].. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes 1. These perhaps, explain why extracts of *Allium sativum* were used in treating wounds and burns [43]. Cardiac glycosides are known to work by inhibiting the Na+/K+pump. This causes an increase in the level of sodium ions in the myocytes, which then lead to a rise in the level of calcium ions. This inhibition increases the amount of Ca2+ ions available for contraction of the heart muscle, which improves cardiac output and reduces distension of the heart; thus they are used in the treatment of congestive heart failure and cardiac arrhythmia [44]. Glycosides are administered in other to promote appetite and aid digestion. They are also used as astringents and as antiprotozoan [1].

Saponins have the ability to cause leakage of proteins and certain enzymes from the cell [45]. It is also used to treat hypercholesterolemia, hyperglycemia; as an antioxidant, anti-cancer, anti-inflammatory and many others [44]

Flavonoids have been found to be effective against a wide array of micro organisms in vitro and are known to be synthesized in response to bacterial infections by plants. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall[46].

Flavonoids are known to have potentially exploitable antibacterial activities, suppression of bacterial influence and synergism with antibiotics [47]. They inhibit a number of bacterial virulence factors including quorum-sensing signal receptors, enzymes and toxins [47]. They are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity[48]. Modern authorized physicians are increasing their use of pure flavonoids to treat many important common diseases; due to their proven ability to inhibit specific enzymes, stimulate some hormones and neuro transmitters and to scavenge for free radicals [49]. Flavonoids, tannins and alkaloids have been shown to have inhibitory effect on prostaglandins synthesis and as compounds with anti¬-inflammatory propertie [50].

The tannin content of *Allium sativum* justifies the use of the extracts from these plants to stop bleeding and in treating wounds [43]. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects [51].

Interestingly, the upper filtrate (the more active fraction) contained more of glycosides, reducing sugars, tannins, flavonoid and saponins. It is possible that these constituents may be contributing to the observed pharmacological effect. These studies are also in agreement with the work of [39], which showed that ethanol extract of *Allium sativum* was more pharmacologically active than the methanol extract.

It has long been known that the extraction of a plant can increase its potency and eliminate unpleasant side effects. The irritating, acidic, oxidizing compounds in garlic can be eliminated or modified by extraction [52].

In our studies, the extraction with ethanol yielded water soluble active constituents with LD_{50} of 3,750 mg/kg (Oral). This compares favourably with the work of [37] which gave the intradermal LD_{50} of *Allium sativum* extract in experimental rabbits as 3,034 mg/kg and maximum tolerated dose of 2,200 mg/kg. Mortality occurred in rabbits given the extract at 3,200 and 4,200 mg/kg with other behavioral signs like loss of appetite and partial paralysis [37]. Alchoholic and aqueous extract of *Allium sativum* contain primarily S-allyl-Cysteine [33]

Inflammation remains a serious health problem in the world today. Current allotropic drugs are showing side effects on prolonged use [53]. The importance of anti-inflammatory agents cannot be over emphasized because of their utility often as life-saving drugs, in many diseases such as arthritis and rheumatoid fever [53]. Clinically anti-inflammatory drugs are judged by their effect on the pain, stiffness or swelling of the affected part, the action on swelling being the most objectively observable, therefore most i[mportant[54].

Inflammation can be induced by a variety of chemical agents which have no coherent and consistent correlation between obtained pharmacological activity and chemical structure [55]. Fresh egg albumin-induced inflammation appear to be similar to carrageanin- induced inflammation in rodents [55].

This study showed that ethanol extract of *Allium sativum* had a dose and time dependent systemic anti-inflammatory effect which is comparable to that of Piroxicam and therefore suggest that there is a potential therapeutic use of ethanol extract of *Allium sativum* in the treatment of chronic inflammatory diseases. This finding is supported by the study of[56], which examined extensively the anti-inflammatory, and immunomodulatory properties *of Allium sativum* and its different bioactive molecules and formulations in invitro/invivo studies.

Evaluation of the anti-microbial properties of ethanol extract of *Allium sativum* showed a statistically significant ($p< 0.05$) and dose dependent inhibitory effect on all test microorganisms when compared with control, and better than Penicillin and Erythromycin except for *Klebsiella pneumonia*. Ethanol extract of *Allium sativum* had a statistically significant ($p< 0.05$) inhibitory effect on all test microorganisms and at all doses tested except for *Klebsiella pneumonia* where inhibition occurred at higher concentration.

In this study, the plant extract was effective against both gram-positive and gram- negative bacteria suggesting the presence of broad spectrum antibiotic compounds. The high antibacterial activity in the ethanol extract of *Allium sativum* may be due to the presence of tannins, saponins, flavonoids, alkaloids, resins and glycosides. These medicinally bioactive components exert anti-microbial action through different mechanisms. This study agrees with the work of [57] , which showed that ethanol extract of *Allium sativum* has a dose dependent inhibitory effect on both gram-positive and gram-negative micro-organisms. Also, [39] in their study showed that ethanol extract of Allium sativum had a dose and time dependent anti-microbial effect against gram-positive and gram-negative microbials.

The broad spectrum antibiotics however recorded significantly higher but comparable anti-microbial activity as the ethanol extract of *Allium sativum*. This showed that ethanol extract of *Allium sativum* posses' anti-microbial compounds which could be used as substitutes for antibiotics or be added to available anti microbials.

5. Conclusion

The presence of flavonoids, resins, alkaloids, saponins, glycosides and many others in the ethanol extract of *Allium sativum* supports the claim that this plant has anti-microbial and anti-inflammatory properties. This is because other plant products containing these constituents have been tested for anti-microbial and anti-inflammatory properties [55].

This study has been able to establish that ethanol extract of *Allium sativum* has a statistically significant ($p< 0.05$) anti-microbial and anti-inflammatory effect in a dose and time-dependent manner, which supports its extensive use as an anti-microbial and anti-inflammatory agent in ethnomedicine.

Further study needs to be done to isolate the active components in the different filtrates of Allium sativum and to elucidate the full anti-microbial and anti-inflammatory profile of *Allium sativum*, as well as the mechanism of action.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the staff and students of MSc Pharmacology and Therapeutics class of 2012/13, Nnamdi Azikiwe University, Nnewi Campus, Nigeria, and to Mr. Augustine Okoli of University of Nigeria, Nsukka who helped in the extraction of the sample.

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

As at the time of this study there were no ethics committee for animal studies\ research works in our centre\ institution.

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