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The Antimicrobial, Anti Inflammatory and Analgesic Activities of the Rhizome Extract of *Curcuma longa* L. (Turmeric)

Ogbonna Abigail Ify[1*], Adejube Glory Raphael[1], Okoye Clifford Tochukwu[1], Ogbonna Ugoy Sonia Amarachi[2], Njoku Andrew Ikechukwu[3], Madu Josephine Madukaihe[4], Yakubu Thomas[5] and Ogbonna Chike Innocent[1]

[1]Department of Plant Science and Biotechnology, University of Jos, Nigeria.
[2]Department of Applied Microbiology & Brewing, Nnamdi Azikiwe University, Nigeria.
[3]Department of Science Laboratory Technology, University of Jos, Nigeria.
[4]Department of Environmental Biotechnology and Bio-conservation, National Biotechnology Development Agency (NABDA), Nigeria.
[5]Department of Pharmacognosy, Faculty of Pharmacy, University of Jos, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author AIO designed the study, wrote the protocol and performed the statistical analysis, Authors AGR, OCT and OUSA wrote the literature review and the first draft of the manuscript. Author OCIC supervised the work. Authors NAI and MJM managed the analysis and editing of the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at investigating the anti-microbial, anti-inflammatory, and analgesic properties of rhizome extracts of *Curcuma longa* (Turmeric).
Methodology: Extraction of biochemical constituents of the 150 g of the turmeric rhizomes powder was carried out successively using hexane, ethyl acetate, methanol, and water as solvents. The antimicrobial potentials of the different fractions of the extract were tested on clinical bacterial

**Corresponding author: E-mail: ogbonabi@yahoo.co.uk;*

including *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumonia* and *Pseudomonas aeruginosa* using agar well diffusion method at concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using agar dilution technique. For the anti-inflammatory effects of the extract of *C. longa*, albino rats were treated with methanolic extract (250, 500, and 1000 mg/kg body weight) and diclofenac as control, after which they were injected with egg albumin on the right hind paw to induce inflammation. The swelling degree of the injected paw was measured. The analgesic effect of *C. longa* methanolic extract on hot plate-induced pain was also investigated in albino rats, and the Index of response latency was recorded.

Results: The biochemical assay showed that *C. longa* extract contained Alkaloids, Saponins, Tannins, Flavonoids, Carbohydrate, Steroids, Terpenes, Anthraquinone, and Cardiac Glycosides, with Methanol and aqueous extracts having the most biochemical constituents. The antibacterial susceptibility screening showed that *K.pneumonia* was the most susceptible organism, having the highest zone of inhibition of 18 mm for ethyl acetate extract at the concentration of 400 mg/ml. This was followed by *S. pneumonia*, *S.aureus* and *P. aeruginosa* with inhibition zone of 13, 12 and 10 mm respectively. The MIC for *K.pneumonia* and *S.aureus* were both 200 mg/ml, while that of *P. aeruginosa* and *S. pneumonia* were both 400 mg/ml. The paw size of rats treated with increasing doses of the extract and diclofenac significantly decreased with time. The concentration of 250 mg/kg had the lowest paw volume of 4.39±0.36mm while 1000 mg/kg recorded 5.18±0.28 as the highest, at 180 minute.

Conclusion: Based on the result obtained, it can be concluded that *Curcuma longa* Rhizome extract possess anti-microbial, anti-inflammatory, and analgesic properties.

Keywords: Antimicrobial; anti-inflammatory; analgesic; rhizome extract; curcuma longa; and albino rats.

1. INTRODUCTION

Medicinal plants serve as sources of drugs in a variety of communities. They are used for the treatment and also in the prevention of several diseases. They are gaining preference due to their low cost and accessibility [1,2]. These plants contain phytochemicals such as flavonoids, alkaloids, glycosides, polyphenols, steriods, tannins, terpenes and many others. They have potentials for use as drugs and have the ability to deter disease or disease-causing microorganisms [3].These phytochemicals provide certain health benefits by promoting or regulating metabolic pathways [4]. Diabetes, cancers, neurological disorders, cardiovascular diseases, inflammations, and other chronic diseases have been treated using medicinal plants over the years and their efficacy in treating these diseases makes them an effective alternative to conventional drugs [5].

Curcuma longa (Turmeric), belonging to the family Zingiberaceae is a perennial herbaceous plant that is native to India and Southeast Asia. It has been reported to possess diverse pharmacological and therapeutic activities [6,7]. Studies have shown that curcumins (Diferuloylmethane), the main bioactive component of turmeric rhizome possess a wide spectrum of biological activities such as antioxidant, hepatoprotective, anti-inflammatory, anti-carcinogenic, anti-microbial, and cardiovascular [8,9].

Medicinal plants or their products have been explored as an alternative to inhibit microbial pathogens because some bacteria and fungi have evolved to produce several defense mechanisms that makes them resistant to conventional drugs [10]. These plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of the plants [11]. A study showed that *C. longa* possess antimicrobial activities against gram positive and negative bacteria and this inhibition might not be solely attributed to curcumins [12]. *Curcuma* oil was tested against cultures of *Staphylococcus albus*, *S. aureus* and *Bacillus typhosus*, and was found to inhibit their growth [13]. Bhavani and Murthy [14] also investigated the activity of turmeric fractions against some intestinal bacteria *in vitro*, and there was total inhibition of growth of Lactobacilli.

The anti-inflammatory activity of any therapeutic agent is of importance in the treatment and prevention of many diseases such as rheumatic fever and arthritis [15]. Inflammation is

characterized by pains and swellings, and the adverse effects that come with allopathic drugs have made many researchers to look inwards for safer and more effective alternatives to treat inflammations [15,10]. The anti-inflammatory effects of curcumin have been established in clinical and experimental studies. However, analog derivatives of curcumins with anti-inflammatory biological activity have also been developed [16,17,18,19]. Khan et al. [12] also reported that methanolic extract of *C. longa* possesses anti-inflammatory activities along with antimicrobial properties.

Pain is an indication of several illnesses or diseases and the treatments requires analgesics [20,21]. Various drugs are available as analgesics but some of these drugs possess adverse effects such as fluid retention, bronchospasms, and gastrointestinal irritation; hence, there is need to discover new drugs with little or no adverse effects [22,23]. Medicinal plants are better alternative to be used as analgesics, as most of them are natural, safe and effective for use [24]. *Curcuma longa* has been reported to possess anti-microbial, anti-inflammatory and analgesic properties [25,26]. There is need for more studies to be carried out on the plant for the discovery of effective potential drugs. The aim of this study is to investigate the anti-microbial, anti-inflammatory, and analgesic activities of the rhizome extract of *Curcuma longa,* which could be useful as a starting point for the discovery of new effective drugs.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh rhizomes of *C. longa* were bought from Farin-gada market, Jos North, Plateau State Nigeria and identification was done in the herbarium of the Department of Plant Science and Biotechnology, University of Jos.

2.2 Preparation of Rhizomes of *C. longa* for Extraction

About 1 kg of fresh rhizomes of *C. longa* was washed under running tap to remove sand and other debris and then were washed in three changes of sterile distilled water. They were sliced and dried in the laboratory using drying cabinet set at 40°C. After drying, the rhizomes were then pulverized into powdered form using clean laboratory mortar and pestle. The powder was then packaged into a well labeled air-tight plastic container and taken to the Laboratory of Department of Pharmacognosy, University of Jos, for extraction of the bioactive agents.

2.3 Extraction of *C. longa* Powdered Plant Material

Serial exhaustive extraction of *C. longa* powdered plant material was carried out according to the method described by Pandey and Tripathi [27]. This method involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. A weight of 150 g of the turmeric rhizomes powder was transferred into a conical flask and soaked with Hexane. The contents were subjected to continuous shaking using mechanical shaker and allowed to stand for 24 hours. The macerate was filtered using clean filter paper on a Buchner funnel flask assisted with a vacuum pump. The residue was subjected to several rinsing and filtration to ensure maximum extraction. The same residue from the above extraction of the plant material was extracted repeatedly using ethyl acetate, methanol, and water respectively. The residue was allowed to dry before addition of the next solvent. The collective filtrates were then evaporated to dryness on a water bath and a rotary evaporator at a controlled temperature in the drying cabinet. The percentage yield of the extract was determined using the formular below and the extracts were then transferred into well labeled sterile sample bottles and were kept in the refrigerator until required.

% Yield of extract = (Final weight of extract/ Original weight of crude powder 1) x 100

2.4 Analysis of *Curcuma longa* Rhizome for Biochemical Components

The analysis of *C. longa* rhizome for biochemical components was done using the method described by Sofowora [28], Banu and Cathrine [29], Trease and Evans [30], to confirm the presence of bioactive components present in the rhizomes of *Curcuma longa.* The rhizome extracts from the various solvents were screened for their biochemical constituents as follows:

2.4.1 Test for alkaloids

A weight of 0.5 g of the extract was stirred with 5 ml of 1 % aqueous Hydrochloric acid on a steam

bath. The solution was filtered and 1 ml of the filtrate was transferred into a test tube. Few drops of Dragendorff's reagent were added to the test tube. An orange-red precipitate confirms the presence of Alkaloids.

2.4.2 Test for saponins

The plant extract (50 mg) was diluted with distilled water (20 ml) and shaken for 15 minutes in a graduated cylinder. The formation of 2 cm thick foam indicated the presence of saponins.

2.4.3 Test for tannins

A weight of 500 mg of the extracts was stirred in 10 ml of distilled water and then filtered. Some drops of 1 % ferric chloride reagent were added to the filtrate (2 ml). A blue-black precipitate was noted as evidence for the presence of tannins.

2.4.4 Test for flavonoids

The extracts were dissolved in water and filtered. 2 ml of 10 % aqueous Sodium Hydroxide was added to the filtrate to produce a yellow coloration. The presence of Flavonoids was observed when the yellow color changes to colorless on addition of dilute Hydrochloric acid.

2.4.5 Test for carbohydrates

The extracts were dissolved in distilled water and mixed with few drops of Molisch reagent. 1 ml of H_2SO_4 was added to the mixture afterwards, and allowed to stand for 2 minutes, which was then diluted with 5 ml of distilled water. Reddish or violet ring at the junction of the liquids confirms the presence of carbohydrates.

2.4.6 Test for steroids

Two ml of the extracts were dissolved in 2ml of chloroform, and 2 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish-brown color produced at the chloroform layer indicates the presence of steroids.

2.4.7 Test for terpenes

One ml of acetic anhydride was added to 2.0 ml of the extract and then 2 drops of concentrated sulphuric acid was carefully added down the side of the test tube. A pink color which changes to bluish green on standing was indicative of the presence of terpenes.

2.4.8 Test for anthraquinones

Borntrager's test was employed for the detection of anthraquinones. Diluted H_2SO_4 was added to 3 ml of each of the extracts. After boiling, the solution was filtered and benzene was added to the cooled filtrate. The solution was shaken vigorously and diluted ammonia solution was added to the organic layer. The ammonia layer turns pink and this confirms the presence of Anthraquinone.

2.4.9 Test for cardiac glycosides

A weight of 0.5g of the extracts were dissolved in 10mls of chloroform in a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1ml of glacial acetic acid containing one drop of Ferric chloride solution. This was then underlaid with 1ml of concentrated Sulphuric acid. A brown ring obtained at the interphase indicated the presence of a deoxy sugar characteristic of cardenolides.

2.5 Collection and Standardization of Test Bacteria

The pure cultures of the test bacteria (*Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumonia* and *Pseudomonas aeruginosa*) were collected from the Microbiology unit of Health Services of University of Jos, Nigeria. The organisms were sub-cultured for the authentication of their identities using standard methods. Modified method of Collins et al. [31] was employed for the standardization of the test bacteria. A loopful of each of the pure isolates was dispensed from broth culture into 10 ml each of the sterile nutrient broth in McCartney bottles which were then incubated at 37°C for 24 hours to standardize the culture to 10^{-5} cfu/ml (McFarland standard). A loopful of the standardized culture was used for the sensitivity testing.

2.6 Antibacterial Sensitivity Testing of *C. longa* against Some Test Bacteria

The antibacterial sensitivity testing of the plant extracts was determined using agar well diffusion method as described by Adeniyi et al. [32]. Using a sterile Pasteur pipette, 0.5 ml of the 10^{-4} dilution of the organisms was pipetted and placed in sterile Petri dishes and 20 ml of already prepared molten nutrient agar was poured into each of the plates and swirled both clockwise and anti clockwise to mix homogenously. The inoculated plates were allowed to stay on the work bench to solidify. A standard sterile cork borer of 6 mm in diameter was used to cut equidistant wells in the agar, into which was added the reconstituted extract of concentrations

400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml respectively. The plates were allowed to stand on the laboratory bench for 1 hour to allow diffusion of the extracts into the medium. The plates which were prepared in triplicates were incubated at 37^{o}C for 24hours. The standard drug gentamycin (40 mg/ml) was used as the control. At the end of the incubation period, diameters of zones of inhibition were measured in millimeters and recorded for the plates that showed susceptibility. All assays were carried out in replicates for accuracy and with their control, and these were done for all the isolates.

2.7 Minimum Inhibitory Concentration (MIC) of the Extract

This is the lowest concentration of an antimicrobial that will inhibit the visible growth of a bacterium. Minimum Inhibitory Concentration (MIC) was determined using agar dilution technique as described by Mebude et al. [33]. Doubling serial dilutions of the extracts were made in different test tubes to give concentrations of 31.25 mg/ml, 15.63 mg/ml, 7.81 mg/ml, 3.91 mg/ml and 1.96 mg/ml. A volume of one ml of the dissolved extract of different concentrations was added to 19 ml of sterile molten nutrient agar. They were properly mixed for even distribution of the extract within the agar medium. The mixtures were poured into sterile Petri dishes and allowed to set. Aliquot of 0.1 ml of each of the test organisms was inoculated into each concentration of agar-extract mixture. This was repeated for all the organisms and each dilution and were all replicated. A positive control plates containing only the growth medium and each of the test organisms were also set up. The whole plates were incubated at 37 ^{o}C for 24 hours. After the incubation period, the plates were examined for the presence of colonies. The lowest concentration that showed no visible growth of any of the test organisms was observed and recorded as the Minimum Inhibitory Concentration (MIC) of the extract for that particular microorganism.

2.8 Minimum Bactericidal Concentration (MBC) of the Extract

This is the minimum effective concentration of bacterial agent that completely kills the bacterium. Minimum Bactericidal Concentration (MBC) was determined using modified method of Mebude et al. [33]. A volume of 0.5 ml of the different concentrations of the extract that did not show visible growth during MIC determination was added to 0.5 ml of test organism in tubes. The tubes were incubated at 25±2 °C for 48 hrs. Aliquots of samples from each tube were cultured on separate Petri dishes containing freshly prepared nutrient agar medium for the determination of the minimum concentration that of the extract required to kill the test organism. The plates were incubated at 37 °C for 24 hrs. The lowest concentration of the extract that shows no visible growth was considered the Minimum Bactericidal Concentration

2.9 Animal Studies

Wistar Albino rats (200 ± 20 g) were supplied by the Experimental Animal house of University of Jos. Animals in various models were used according to the principle of half male and half female except for only female rat in hot-plate test. The animals were allowed free access to food (growers mash) and water under a 12h/12-hour light/dark cycle with the room temperature maintained at 25 ± 2 °C and relative humidity of 40-60%. The study protocols were approved by the Ethics Committee on Animal Experiment, University of Jos.Toxicity study was done to determine the LD_{50} (Lethal Dose) which was found to be greater than 5000 mg/ml (LD_{50} > 5000 mg/ml) indica ting that the extract is not toxic.

2.10 Anti-Inflammatory Activities of *Curcuma longa*

The anti-inflammatory activity was carried out according to the method described by Jun et al. [34]. Twenty-five adult male rats were randomly divided into five groups of five rats each. Group 1 rats (negative control) were administered normal saline (5 ml/kg body weight), Group 2 (positive control) received Diclofenac (25 mg/kg), Groups 3-5 received turmeric extracts (250, 500, and 1000 mg/kg body weight respectively). All treatments were orally administered using oral canula. Test drugs (Diclofenac and turmeric extracts) were administered to 24 hour fasted rats 1 hour before the induction of inflammation. The right hind paw size of the rats at time zero (before the induction of oedema) was measured using a vernier caliper. The phlogistic agent employed to induce acute inflammation in the rats was fresh egg albumin (0.1 ml of 20% in normal saline) injected into the sub plantar tissue of the right hind paw. The swelling degree of the injected paw was measured at 0, 30, 60, 90, 120,

150, and 180 mins after the administration of the phlogistic agent (egg albumin). The average oedema at every interval was assessed in terms of difference in volume displacement after injecting the egg albumin and zero-time (basal paw) volume displacement of the injected paw (Vt-Vo). Percentage inhibition of oedema was calculated for each treated group using the formula:

$$\text{Percentage inhibition of edema} = \frac{V_o - V_t}{V_o} \times \frac{100}{1}$$

Where V_o and V_t are edema volume of negative control and treated groups, respectively at the corresponding time, t.

2.11 Analgesic Effect of *Curcuma longa* by Hot-Plate Test

In the determination of analgesic effects of *C. longa*, the effects of the extract on hot plate-induced pain were investigated in albino rats. The hot plate test was used to measure response latencies according to the method described by Meng et al. [35]. The animals were randomly divided into five groups: Group 1 served as negative control and received normal saline (5 ml/kg), Group 2 served as positive control and received Pentazocine (20 mg/kg), Groups 3, 4 and 5 were pretreated with turmeric extract (250, 500, and 1000 mg/kg) respectively 30 mins prior to the placement on the hot plate. Each rat was placed in a glass beaker of 50 cm and kept on a hot plate having a constant temperature of 50 ± 5°C. Index of response latency was recorded as the time(s) between placement and licking of the hind paw or jumping out of the hot plate by each animal. The nociceptive response was measured 30 min after treatment and every 30 min for 3hrs. The increase in latency time in relation to the initial time for each group was taken as an index of analgesic activity.

2.12 Statistical Analysis

Statistical analysis was carried out by subjecting data to Analysis of Variance (ANOVA) followed by a Tukey-Kramer comparison test. Results were represented as mean ± SEM and at $P < 0.05$, the results were considered statistically significant.

3. RESULTS

3.1 Percentage Yield of the Extracts

The percentage yield of hexane, methanol, ethyl acetate and aqueous extracts of rhizome of *C. longa* were recorded to be 5.73 %, 8.2 %, 8.87 % and 11.6 % respectively, indicating that the aqueous extract yielded more, while hexane yielded the least. The result is shown in Table 1.

3.2 Biochemical Constituents of Rhizome Extract of *Curcuma longa*

The analysis of the rhizomes extracts of *Curcuma longa* for biochemical components reveals that the rhizome contained various biochemical constituents which included Alkaloids, Saponins, Tannins, Flavonoids, Carbohydrate, Steroids, Terpenes, Anthraquinone, and Cardiac Glycosides. Of the four extracts, methanolic and aqueous fractions were found to contain more of the biochemical constituents than the Hexane and Ethyl acetate extracts. Tannins were found to be present only in the methanolic extract while alkaloids and saponins were observed only in the aqueous extract. Steroids and cardiac glycosides were found to be present in all four (4) extracts. The results are presented in Table 2.

3.3 Antibacterial Sensitivity Screening of the Different Solvent Extracts of *C. longa*

The antibacterial sensitivity screening of the different solvent extracts of *Curcuma longa* showed that they all had activities on the clinical isolates employed in the study. *K. pneumonia* was the most susceptible organism, having the highest zone of inhibition of 18 mm for ethyl acetate extract at the concentration of 400 mg/ml. This was followed by *S. pneumonia*, *S. aureus,* and *P. aeruginosa* with inhibition zone of 13, 12 and 10 mm respectively as shown in Tables 3a-3d. The extracts from the other solvents showed inhibitions but these were not as effective on the different isolates as that of ethyl acetate. However, all the organisms were inhibited by the standard drug (Gentamycin 4 mg/ml) with *K. pneumonia* having the highest zone of inhibitions of 20 mm.

The other extracts of *C. longa* from hexane and aqueous fractions followed the same trend with *K. pneumonia* having the highest zone of inhibition except for the methanol fraction that had *S. aureus* with highest zone of inhibition of 14.5 mm and then *K. pneumonia* with 10.4 mm. The inhibition zones recorded for all the test bacteria were dose dependent showing that the higher the concentration, the higher the zone of clearance. The standard drug had higher effects on the test bacteria with *K. pneumonia* being the

most susceptible recording 20 mm zone of inhibition. This was followed by *S. pneumonia, P. aeruginosa* and *S. aureus* recording 17, 14 and 14.5 mm respectively. For the methanolic extracts, *S. aureus* competed favorably with the standard drug with zone of inhibition of 14.5 and 14 mm respectively. The details of results are presented in Tables 3a- 3d.

Table 1. Percentage yield of hexane, methanol, ethyl acetate, and aqueous *c. longa* extracts

Extract	Weight (g)	Percentage yield (%)
Hexane	150	5.73
Methanol	150	8.2
Ethyl acetate	150	8.87
Aqueous	150	11.6

Table 2. Phytochemicals present in *C. longa* (Turmeric) rhizomes extract extracts

Phytochemicals	Hexane	Methanol	Ethyl Acetate	Aqueous
Alkaloids	-	-	-	+
Saponins	-	-	-	+
Tannins	-	+	-	-
Flavonoids	-	+	-	+
Carbohydrate	-	+	-	+
Steroids	+	+	+	+
Terpenes	-	+	-	-
Anthraquinones	+	+	+	-
Cardiac Glycosides	+	+	+	+

+ = Present - = Absent

Table 3a. Antibacterial activity of *C. longa* Rhizome extract against *Klebsiella pneumonia*

Conc. (mg/ml)	Aqueous	Zone of Methanol	Inhibition (mm) Ethyl Acetate	Hexane
400	14.00±1.00	10.40±1.97	18.00±2.00	10.00±1.00
200	12.5±1.80	9.60±0.69	15.00±4.36	8.30±1.81
100	8.33±2.08	8.50±1.50	12.00±2.65	7.17±1.61
50	7.00±2.65	8.67±1.53	9.00±1.73	7.00±1.00
25	6.00±1.00	8.00±1.73	7.00±1.73	6.00±1.73
Gentamycin	20.67±1.53			

Table 3b. Antibacterial activity of *C. longa* Rhizome extract against *Streptococcus pneumonia*

Conc. (mg/ml)	Aqueous	Zone of Methanol	Inhibition (mm) Ethyl Acetate	Hexane
400	10.00±2.00	8.00±1.73	13.00±1.73	10.00±1.73
200	8.50±1.80	6.00±2.00	11.00±1.00	7.53±1.29
100	7.00±1.73	0.00±0.00	9.00±1.73	7.40±1.22
50	6.00±2.00	0.00±0.00	8.50±1.50	7.00±1.41
25	0.00±0.00	0.00±0.00	7.00±1.00	6.00±1.73
Gentamycin	17.00±1.00			

Table 3c. Antibacterial activity of the extracts of *C. longa* Rhizome against *Staphylococcus aureus*

Conc. (mg/ml)	Aqueous	Zone of Methanol	Inhibition (mm) Ethyl Acetate	Hexane
400	0.00±0.00	14.50±2.17	12.00±1.00	7.50±1.32
200	0.00±0.00	13.00±1.73	10.00±2.00	6.00±2.65
100	0.00±0.00	10.00±1.00	9.00±1.73	0.00±0.00
50	0.00±0.00	7.00±1.41	8.00±1.73	0.00±0.00
25	0.00±0.00	6.00±1.00	6.00±1.00	0.00±0.00
Gentamycin	14.00±1.00			

Table 3d. Antibacterial activity of the extracts of *C. longa* Rhizome against *Pseudomonas aeruginosa*

Conc. (mg/ml)	Aqueous	Zone of Methanol	Inhibition (mm) Ethyl Acetate	Hexane
400	8.00±1.00	0.00±0.00	10.00±1.73	8.00±1.00
200	6.00±1.00	0.00±0.00	8.67±2.08	6.00±1.00
100	0.00±0.00	0.00±0.00	7.50±0.50	0.00±0.00
50	0.00±0.00	0.00±0.00	7.00±1.73	0.00±0.00
25	0.00±0.00	0.00±0.00	6.00±1,73	0.00±0.00
Gentamycin	14.00±1.73			

3.4 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Different Fractions of *C. longa* on the Test Organisms

The Minimum Inhibitory Concentration (MIC) of the hexane extract of *C. longa* on *K. pneumonia* and *S. aureus* was 200 mg/ml respectively while that of *P. aeruginosa* and *S. pneumonia* was 400 mg/ml. The Minimum Bactericidal Concentration (MBC) for the hexane extract was 400 mg/ml for both *K. pneumonia* and *S. aureus* and was >400 mg/ml for *P. aeruginosa* and *S. pneumonia*. For the methanol fraction, the MIC recorded for *K. pneumonia* was 25 mg/ml. *S. aureus* and *S. pneumonia* recorded 200 mg/ml and *P. aeruginosa* recorded MIC of 400 mg/ml. The MBC for the test organisms was 50 mg/ml for *K. pneumonia,* 400 mg/ml for *S. aureus* and *S. pneumonia* while that of *P. aeruginosa* was above 400 mg/ml. The details of the results of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration are presented in Tables 4a-4d.

3.5 Anti-inflammatory Activity of Extract of *Curcuma longa* on Egg Albumin-Induced Paw Oedema in Albino rats

The Anti-inflammatory studies on the effects of the extract of *C. longa* on egg albumin-induced rat paw oedema showed that the different concentrations of the extract significantly ($P \leq 0.05$) inhibited oedema formation from 30 mins-180 mins when compared to the negative control. There were no statistically significant ($P \geq 0.05$) reductions in the mean paw oedema of rats in the negative control group at the different time intervals. The paw size of rats treated with increasing doses of the extract and diclofenac sodium significantly decreased with time. The anti-inflammatory activities of the different concentrations (250, 500 and 1000 mg/ml) of the extract were found to suppress the development of paw oedema induced by egg albumin in rats in a dose-dependent manner comparable to diclofenac sodium, the standard drug. The lowest concentration (250 mg/ml) of the extract produced maximum inhibition by producing the lowest paw oedema volume of 4.39±0.36, thereby significantly inhibited ($p \leq 0.05$) paw oedema after 3 hrs (180 mins) of observation as compared to the positive control group with paw oedema volume of 4.68±0.45. The details of the results of anti-inflammatory effects of *C. longa* on egg albumin-induced paw oedema in albino rats are shown in Table 5.

3.6 Analgesic Effects of *Curcuma longa* on Albino ratsby Hot-Plate Test

The results of the analgesic effects of *C. longa* on albino rats by hot-plate test showed that the extract exhibited a dose-dependent increase in the latency response in the hot-plate test. The increases in latency responses (analgesic effects) were found to be statistically significant ($P \leq 0.05$) when compared to the control used. The highest concentration (1000 mg/ml) of the extract produced the highest latency response of 15.80±0.37. The 500, 200, pentazocine and normal saline produced latency responses of 15.14±0.21, 9.30±0.19, 4.98±0.08 and 4.70±0.23 respectively at 180 mins. The details of the results of analgesic effects of the *C. longa* extract on albino rats are presented in Table 6.

4. DISCUSSION

The yield of the *C. longa* extract varied with the extraction solvents used in the study. Aqueous extract showed the highest extractive value, 11.6% followed by ethyl acetate, methanol and Hexane with the percentage yield of 8.87%, 8.2% and 5.73% respectively (Table 1). The high yield of aqueous extract showed that the solvent (water) facilitated the extraction of bioactive chemicals that are soluble in water and also

shows that the rhizome of *C. longa* contains mostly polar components. The aqueous solvent was able to isolate about six bioactive components including Alkaloids, Saponins, Flavonoids, Carbohydrates, Steroids and Cardiac Glycosides. It was observed that methanol, another polar solvent extracted about seven bioactive chemicals including Tannins, Flavonoids, Carbohydrate, Steroids, Terpenes, Anthraquinones and Cardiac Glycosides. Though ethyl acetate and hexane had percentage yield of 8.87% and 5.73% respectively, they could extract only three bioactive components which included Steroids, Anthraquinones and Cardiac Glycosides. The extractive values of all the fractions of the rhizome did not affect the antibacterial potential of the extracts. The results of the yield of *C. longa* are similar to that of Sulaiman et al. [36] where the effect of extraction solvent on the Phytoconstituents of *Aegle marmalos* showed maximum yield for hydro-alcohol extract compared to other extracts. The authors also reported that yield of extract varies with extraction solvent and the part of the plant used. Mujtaba et al. [37] also reported highest yield of extract with 70% methanol in their work on Effect of Solvents on extraction yield, total Flavonoid, Total Phenolic contents, DPPH scavenging activity and antibacterial potential of three Apricot Cultivars. Other reports on efficiency and polarity of solvent are influenced by several parameters such as nature of phytochemicals, the method used for extraction, particle size, and the solvent used [38,39,40]. The exhaustive extraction employed helped in the extraction of many of the bioactive constituents in the plant under study. Biologically active compounds usually occur in low concentration in plants [41]. Extraction technique is that which is able to obtain extracts with high yield and with minimal or no changes to the functional properties of the extract. Many authors reported variations in the biological activities of extracts prepared using different extraction techniques. It is therefore necessary to select the suitable extraction method as well as solvent based on sample matrix properties, efficiency and desired properties [42,43,44].

Table 4a. MIC and MBC for hexane extract of *C. longa* on test bacteria

Organisms	**Concentration (mg/ml)**						
	400	200	100	50	25	MIC	MBC
Klebsiella pneumonia	-	-	+	+	+	200	400
Pseudomonas aeruginosa	-	+	+	+	+	400	>400
Staphylococcus aureus	-	-	+	+	+	200	400
Streptococcus pneumonia	-	+	+	+	+	400	>400

+ = growth - = no growth

Table 4b. MIC and MBC for methanol extract of *C. longa* on test bacteria

Organisms	**Concentration (mg/ml)**						
	400	200	100	50	25	MIC	MBC
Klebsiella pneumonia	-	-	-	-	-	25	5
Pseudomonas aeruginosa	+	+	+	+	+	400	>400
Staphylococcus aureus	-	-	+	+	+	200	400
Streptococcus pneumonia	-	-	+	+	+	200	400

+ = growth - = no growth

Table 4c. MIC and MBC for ethyl acetate extract of *C. longa* on test bacteria

Organisms	**Concentration (mg/ml)**						
	400	200	100	50	25	MIC	MBC
Klebsiella pneumonia	-	-	-	-	-	25	50
Pseudomonas aeruginosa	-	-	-	-	+	50	100
Staphylococcus aureus	-	-	-	-	-	25	5
Streptococcus pneumonia	-	-	-	-	-	25	50

+ = growth - = no growth

Table 4d. MIC and MBC for aqueous extract of *C. longa* on test bacteria

Organisms	Concentration (mg/ml)						
	400	200	100	50	25	MIC	MBC
Klebsiella pneumonia	-	-	-	+	+	100	200
Pseudomonas aeruginosa	-	-	+	+	+	200	400
Staphylococcus aureus	+	+	+	+	+	>400	>400
Streptococcus pneumonia	-	-	-	-	+	50	100

+ = growth - = no growth

The antibacterial susceptibility testing of the extracts from different solvents revealed that the pathogenic bacteria were susceptible to the extracts especially at higher concentration except for aqueous and methanol extracts that did not have effects on *S. aureus* and *P. aeruginosa* respectively for all the concentrations. *K. pneumonia* was the most susceptible organism, having the highest zone of inhibition of 18 mm for ethyl acetate extract at the concentration of 400 mg/ml. The same organism recorded 14 and 10 mm each for aqueous, methanol and hexane fractions respectively at the same concentration (Table 3a). The high zone of inhibition of 18 mg/ml recorded for ethyl acetate fraction competed favorably with that of the standard drug (gentamycin) 20.67 mm. The other test bacteria, *S. pneumonia, S. aureus* and *P. aeruginosa* were also found to be more susceptible to ethyl acetate fraction more than extracts from the other solvents recording 13, 12 and 10 mg/ml respectively as well as those of the control drug, gentamycin (Table 3b-3d). These results obtained indicated that the ethyl acetate fraction of *C. longa* could be employed in treatment of localized infections and septicemia caused by *S. aureus*. This study agrees with that carried out by Khan et al. [12], who investigated the anti-microbial activities of *C. longa* methanolic extract whereby it inhibited 14 bacterial species, in a dose-dependent manner. The results obtained from the antibacterial susceptibility testing showed that at the various concentrations used in the study, the inhibition diameters of the bacterial growth for a given species were lower than those of gentamycin. The distinct sensitivity of the species to the different extracts could be due to the intrinsic features specific to each bacterium such as permeability of the cell wall, presence of an external membrane and with the biochemical profile of the extracts [44]. Abdullah et al. [45] also reported that hydrophobicity is an important property of plant extracts which enables them to rupture cell membrane and remove vital molecules and ions of bacteria out of the cells and ultimately kill bacteria by forming bonds to the lipid layer of the cell membrane of bacteria and mitochondria.The aqueous extract also exhibited high activity on *K. pneumonia* and *S. pneumonia* recording inhibition zones of 14 and 10 mm respectively (Table 3a and 3b). This could have stemmed from the fact that the yield of the aqueous extract was high; this result agrees with the work of Takon et al. [46]. The extract was more effective on gram negative test bacteria used. Though the aqueous extract also inhibited *S. pneumonia* which is a gram-positive strain, it was not effective on *S. aureus* in all the concentrations used. The extracts could be said to have broad spectrum activity and could be potential source of antimicrobial agents for the treatment of pathogenic infections.

The results of the MIC and MBC of rhizome extract of *C. longa*showed bactericidal properties against all the pathogenic test bacteria used (Tables 4a-4d). The inhibitory effects of the extracts of *C. longa* are likely due to the bioactive principles found in the extract which must have worked singly or synergistically, and these bioactive compounds have been reported by several authors for their antibacterial activities and have been employed in modern medicine for drug development [47]. Several molecules isolated from plants such as pinocembrine, ponciretine, sophora flavanone G and naringineare attributed to the antimicrobial activities in both Gram positive and Gram-negative bacteria [47].

The present study also investigated the *in vivo* anti-inflammatory activity of the methanol fraction of the rhizome extract of *Curcuma longa*. The anti-inflammatory potential was investigated by its ability to reduce local oedema induced in the albino rat paw by injection of an irritant or phlogistic agent which in this case was egg-albumin [48]. The extract at 250, 500 and 1000 mg/kg body weight showed a good anti-inflammatory activity as it significantly ($P \leq 0.05$) inhibited the increase in paw volume from 0.5 to 3 hour (30 -180 mins), compared to the control. The anti-inflammatory activity of the extract was

Table 5. Anti-Inflammatory activity of extract of *C. longa* on egg albumin-induced paw oedema in albino rats mean paw oedema (mm) at different time intervals (mins)

Treatment	0min	30mins	60mins	90mins	120mins	150mins	180mins	Total
Normal Saline (5ml/kg)	5.12±0.11	7.72±1.17	7.10±1.14	6.84±1.37	6.62±1.35	6.69±0.93	6.43±1.11	6.65^{a}
Diclofenac (25mg/kg)	5.12±0.58	6.69±0.87	6.36±0.69	5.84±0.67	5.27±0.17	5.10±0.04	4.68±0.45	5.58^{cd}
Turmeric Extract (250mg/kg)	5.13±0.27	6.05±0.17	5.91±0.20	5.56±0.29	5.18±0.30	4.84±0.46	4.39±0.36	5.29^{e}
Turmeric Extract (500mg/kg)	5.47±0.60	6.70±0.90	6.64±0.81	6.38±0.79	5.76±0.35	5.71±0.50	5.48±0.47	6.02^{b}
Turmeric Extract (1000mg/kg)	4.81±0.57	6.80±0.67	6.49±0.19	6.10±0.63	5.75±0.72	5.26±0.22	5.18±0.28	5.77^{bc}

n=5 Mean±SDColumn having the different superscripts are significantly different (P ≤0.05)

Table 6. Analgesic activity of *c. longa* on thermally induced pain in albino rats using hot plate test

Treatments	Mean latency response (seconds) at different Time (mins) Intervals						
	30min	60mins	90mins	120mins	150mins	180mins	Total
Normal Saline (5ml/kg)	4.88±0.08	4.74±0.25	4.62±0.24	4.54±0.27	4.66±0.23	4.70±0.23	4.69^{e}
Pentazocine (20mg/kg)	5.26±0.49	5.06±0.18	5.04±0.09	5.06±0.15	5.02±0.08	4.98±0.08	5.07^{d}
Turmeric extract (250mg/kg)	9.50±0.41	9.56±0.44	9.64±0.23	9.22±0.87	9.48±0.38	9.30±0.19	9.45^{c}
Turmeric Extract (500mg/kg)	14.76±0.60	14.92±0.62	15.20±0.26	15.40±0.21	15.12±0.23	15.14±0.21	15.09^{b}
Turmeric Extract (1000mg/kg)	15.38±1.64	15.28±0.71	15.48±0.43	15.62±0.36	15.90±0.55	15.80±0.37	15.58^{a}

n=5 Mean±SDColumn having the different superscripts are significantly different (P ≤0.05)

found to suppress the development of paw oedema induced by egg albumin in rats in a dose-dependent manner comparable to diclofenac. This suggests that the extract possibly acted by inhibiting the release of vasoactive substances (histamine, serotonin, and kinins) and prostaglandins, as study has showed that Histamine induces paw oedema in rats by evoking the release of prostaglandins and inflammatory mediators [34]. The extract administered at a dose of 250 mg/kg body weight exhibited a potent anti-inflammatory effect by producing a maximum inhibition in paw volume (5.29 cm) and significantly ($P \leq 0.05$) inhibited egg albumin induced paw oedema after 3 hours of observation compared to the diclofenac control group (5.58 cm maximum inhibition). There was no statistically significant difference between the groups administered with 500 and 1000 mg/kg body weightthroughout the 3 hours of observation. This could be attributed to the fact that the methanolic extract of the plant is as stable as the control drug, diclofenac or could even have a longer shelf-life. This result is in agreement of the findings of Obonga et al. [49] in their work on the evaluation of antioxidant and anti-inflammatory properties of *Marantochloa leucantha* extracts and factions both *in vivo* and *in vitro*. Many reports have it that egg-albumin or carrageenan subcutaneous injection into the rat paw produces oedema from plasma protein-rich fluid exudation. Oedema induced by a phlogistic agent has three distinct phases based on the principle of release of various inflammatory mediators. The first phase (0-90 mins) is predominantly non-phagocytic and is mainly mediated by histamine and serotonin, the second phase (90-150 mins) is mediated by kinin and the last phase (150-360 mins) is due to the liberation of Prostaglandins PGs which produces oedema dependent on neutrophils mobilization [49,50,51]. The results showed that the extract not only inhibited the first and the second phases of the inflammatory response but also inhibited the oedema up to 3 hours (the last phase), probably by inhibiting prostaglandin synthesis [52].

The suppression of oedema in the second and third phase of inflammation suggests that the anti-inflammatory activity of the extract may also be due to the suppression of kinin and prostaglandin formation induced by egg-albumin within this period. Since these mediators cause oedema by increasing vasodilatation and vascular permeability at the site of injury, the extract therefore reduces vascular permeability and fluid exudation, thus, suppressing oedema; this could probably be due to the fact that the methanol fraction of the extract was rich in flavonoids, which have been reported to exhibit anti-inflammatory effects by inhibiting the enzyme prostaglandin synthase and more specifically the endoperoxidase [53,54]. The preliminary biochemical screening of the methanolic extract of *C. longa* in addition to flavonoids also contained tannins, steroids, carbohydrates, terpenes, anthraquinones and cardiac glycosides which must have worked singly, complementary or in synergy to provide the observed anti-inflammatory activities.

The findings of this research also showed that the methanolic extract of *C. longa* possess analgesic activity in the hot-plate method used. The rats pretreated with *C. longa* extract showed increase in latency response in a dose dependent manner (Table 6). It was observed that the latency response reached a maximum at time interval of 30 minutes with increase over time. The pain threshold inhibition was significantly ($P \leq 0.05$) shown at doses of 500 and 1000 mg/kg of the extract and was comparable to those of the positive control, pentazocine. At 90-minute, *C. longa* extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight indicated latency periods of 9.64, 15.20 and 15.48 seconds respectively showing pain inhibition. The control drug, pentazocine had 5.04 seconds. The findings showed that the extract at various concentrations (doses) inhibited pain stimulus by having prolonged latency period better than the negative and positive control. The work is in agreement with the work of Shorinwa et al. [55] on anti-inflammatory and analgesic activities of methanol extracts of stem bark of *Anthocleista djalonensis* in wistar rats.

The observed analgesic properties of the methanolic extract of this plant could be attributed to the biochemical components of the plants such as tannins, flavonoids and terpenes. Flavonoids have been reported to have antioxidant, anti-inflammatory and analgesic properties which could be as a result of their inhibitory effects on the enzymes that are involved in the production of chemical mediators of inflammation and pain perception [56,57,58,59]. It has been established that flavonoids directly inhibit prostaglandins that could cause pain and inflammation [60]. The precursor releases arachidonic acid through cyclooxygenase and prostaglandin biosynthesis which play roles in the nociceptive mechanism

[61]. This therefore shows that the inhibition of acute inflammation by the extract leads to their inhibitory effects on pain development process [61,62,63]. The findings of this research work have shown that the pharmacological activities of the *C. longa* extract had provided the evidence for the use of the plant in the treatment and prevention of microorganisms, inflammations, and pain caused by different types of oxidative stress including cancer, arthritis and pain of different kinds.

5. CONCLUSION

The fractions of *C. longa* from Ethyl acetate, methanol, hexane and aqueous solvents were effective on the pathogenic test bacteria used in this study but were not as effective when compared with Gentamycin. The methanolic extract of the rhizome was also effective as anti-inflammatory and analgesic agents against laboratory models. This study confirms the efficacy of *C. longa* in the treatment of various kinds of diseases as scientific bases for its traditional uses. The above description in this research work proves that curcumin has enormous potential for treatment of a variety of diseases. The potential of this plant must be explored more and more, in order to develop an alternate therapy for the treatment of infections caused by antibiotic-resistant bacteria and oxidative stress caused by free radicals.

DISCLAIMER

The products used for this research are commonly and predominantly used laboratory products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The study protocols were approved by the Ethics Committee on Animal Experiments, University of Jos. The Ethical clearance Reference/ID number is UJ/FPS/F17-00379.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mintah SO, Asafo-Agyei T, Archer MA, Junior PA, Boamah D, Kumadoh D, Appiah A. Oxtrcloo A. Boakye Y D, Agyare C. Medicinal Plants for Treatment of Prevalent Diseases. In Pharmacognosy-Medicinal Plants; Intech Open:London, UK, 2019.
DOI:10.5772/intechopen.82049.
2. Asase A, Kokubun T, Grayer RJ, Kite G, Simmonds MSJ, Oteng-Yeboah AA, et al. Chemical constituents and antimicrobial activity of medicinal plants from Ghana: Cassia sieberiana, Haematostaphisbarteri, Mitragynainermis and Pseudocedrelakotschyi. Phytotherapy Research. 2008;22(8):1013-1016.
3. Ahn K. The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB Reports. 2017; 50(3) 111–116. DOI:10.5483/BMBRep.2017;50(3)221.
PMC 5422022
PMID 27998396
4. Abuajah CI, Ogbonna AC, Osuji CM. Functional components and medicinal properties of food: a review. Journal of Food Science and Technology. 2004;52:2522–2529.
DOI: 10.1007/s13197-014-1396-5
5. Mohammed E, Chattopadhyay D, Feo V, Cho WC. Medicinal Plants in the Prevention and Treatment of Chronic Diseases. Evidence-Based Complementary and Alternative Medicine. 2014;3.
DOI: 10.1155/2014/180981.Article ID 180981

6. Jana L, Otakar S, Sirtl W, Karol M. On the identity of turmeric: the typification of Curcuma longa L. (Zingiberaceae). Botanical Journal of the Linnean Society. 2008;157(1):37–46. DOI:10.1111/j.1095-8339.2008.00788.x
7. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. The International Journal of Biochemistry & Cell Biology.2009;41:4059.
8. Gupta B, Ghosh B. Curcuma longa inhibits TNF-alpha induced expression of adhesion molecules on human umbilical vein endothelial cells. International Journal Immunopharmacology. 1999;21(11):745-57.
9. Liju VB, Jeena K, Kuttan R. An evaluation of antioxidant, anti-inflammatory, and antinociceptive activities of essential oil from Curcuma longa. L. Indian Journal of Pharmacology. 2011;43(5): 526-531.
10. Anwar A, Rafiq S, Rasool ST, Asif AH. Anti-inflammatory and antimicrobial activity of V. aconitifolia. Academia Journal of Medicinal Plants.2018; 6(11):347- 352. DOI: 10.15413/ajmp.2018.0128
11. Nascimento G, Locatelli P, Freitas C, Silva G. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic resistant Bacteria. Brazilian Journal of Microbiology 2000;31:247-256.
12. Khan MB, Rabby A, Ullah H, Hossain CF. Investigation of Antimicrobial and Anti-inflammatory Activity pf Curcuma longa. International Journal of Pharmaceutical Sciences and Research. 2013; 4(3):1105-1109.
13. Araújo CC, Leon LL. Biological activities of Curcuma longa L.Memorias do Instituto Oswaldo Cruz 2001;96:723-728.
14. Bhavani TN, Murthy S. Effect of Turmeric (Curcuma longa) fractions on the growth of some intestinal and pathogenic bacteria in vitro. Indian Journal Experimental Biology. 1979; 17: 1363-1366.
15. Lalsare S, Verma PK, Khatak M, Ranjan S, Rajurakar S, GuravS. Anti-Inflammatory and Antimicrobial activity of FlacourtiaRamontchi Leaves. International Journal of Drug Development & Research. 2011;3(2):308-313.
16. Jacob A, Wu R, Zhou M, Wang P. Mechanism of the anti-inflammatory effect of curcumin: PPAR-γ activation. PPAR Research.2007;69–73.
17. Kumar P, Padi SS, Naidu PS, KumarA.Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. Methods For Experimental Clinical Pharmacology. 2007;29:1925.
18. Hemeida RA, Mohafez OM. Curcumin attenuates methotraxate induced hepatic oxidative damage in rats. Journal Egypt Natural Cancer Institute. 2008-20:141-148.
19. Zhang Y, Liang D, Dong L, Ge X, Xu F, Chen W. Anti-inflammatory effects of novel curcumin analogs in experimental acute lung injury. Respiratory Research. 2015;16:4355.
20. Jain KN, Kulkarni KS, Singh A. Modulation of NSAID-induced antinociceptive and anti-inflammatory effects by alpha2-adrenoceptor agonists with gastroprotective effects. Life Sciences. 2002;70:2857–2869. DOI:1016/S0024-3205(02)01549-7
21. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. European Journal of Medicinal Chemistry. 2007;42:125–137. DOI: 10.1016/j.ejmech.2006.09.019
22. Osadebe PO, Okoye FBC. Anti-inflammatory effects of crude methanolic extract and fractions of Alchorneacordi folia leaves. Journal of Ethnopharmacology. 2003;89:19–24. DOI:10.1016/S0378-8741(03)00195-8
23. Raza M, Shaheen F, Choudhary MI, Suria A, Rahman UA, Sompong S, Delorenzo RJ. Anticonvulsant activities of the FS-1 subfraction isolated from roots of Delphinium denudatum. Phytotherapy Research. 2001;15:426–430. DOI:10.1002/ptr.792
24. Efferth T, Kuete V. Cameroonian medicinal plants: Pharmacology and derived natural products. Frontiers in Pharmacology. 2010;1:123.
25. Jogdand S, Bhattacharjee J. Evaluation of analgesic activity of turmeric (Curcuma longa Linn.) in Wister rats. International Journal of Basic & Clinical Pharmacology. 2017;6(3). DOI:10.18203/2319-2003.ijbcp20170814
26. Yimam M, LeeYC, Moore B, JiaoP, Hong M, Nam JB, et al., Analgesic and anti-inflammatory effects of UP1304, a botanical composite containing standardized extracts of Curcuma longa

and Morus alba. Journal of integrative medicine.2016;14(1):60–68. DOI:10.1016/S2095-4964(16)60231-5
27. Pandey A. Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014; 2(5):115-119.
28. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 3rd Edition. Ibadan, Nigeria: Spectrum Books Limited. 2008;199-204.
29. Banu KS, Cathrine L. General Techniques Involved in Phytochemical Analysis. International Journal of Advanced Research in Chemical Science. 2015;2(4):25-32.
30. 30. Trease GE, Evans WC. Pharmacognosy. 15th Edition London: W.B. Sanders. 2002;183-393.
31. Collins CH, Lyne PM, Grange JM, Falkinham JO. Microbiological Methods. 8th Edition. Arnold, 338 Euston Road, London NW1 3BH;2004.
32. Adeniyi BA, Ayepola OO. The Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of Eucalyptus camaldulensis and Eucalyptus torelliana (Myrtaceae). Research Journal of Medicinal Plants 2008;2:34-38.
33. Mebude OO, Temitope O, Lawal,Bola AA. Anti-Dermatophytic Potential of Formulated Extract of Cola nitida (Stem Bark). Journal of Clinical and Experimental Dermatology Research 2017;8:3. DOI:10.4172/2155-9554.1000379394
34. Jun Z, Aosimanjiang M, Chenyang L, Qian L, Fang X, Tao L. . Evaluation on Analgesic and Anti-Inflammatory Activities of Total Flavonoids from Juniperus sabina. Evidence-Based Complementary and Alternative Medicine. ID 7965306, 2018;9. DOI:10.1155/2018/7965306
35. Meng LD, Wang L, Du JN. "The analgesic activities of *Stauntonia brachyanthera* and YM11 through regulating inflammatory mediators and directly controlling the sodium channel prompt." Scientific Reports 2017;7(1).
36. Sulaiman CT, Shahida V, Balachandran I. Effect of Extraction Solvent on the Phytoconstituents of Aegle marmelos. Journal of Natural Remedies 2015:15(1):58-64.
37. Mujtaba A, Masud T, Ahmad A, Naqvi SM, Qazalbash MA., Levin RE. Effect of Solvents on Extraction Yield, Total Flavonoid, Total Phenolic Contents, DPPH Scavenging Activity and Antibacterial Potential of Three Apricot Cultivars. Transylvanian Review 2016;24(10):1662-1676.
38. Sultana B, Anwar F, Ashraf M. Effect of Extraction Solvent andTechnique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules 2009;14: 216780.
39. Shabbir G, Anwar F, Sultana B, Khalid ZM, Afzal M, Khan MQ, Ashrafuzzaman M. Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold 1676 Mohar [Delonix regia (Bojer ex Hook.) Raf.]. Molecules 2011;16:7302-7319.
40. Do QD, Angkawijaya AE, Tran-Nguyen PL., Huyn LH, Soetaredjo FE., IsmadjiS., et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatic. Journal of food and drug analysis. 2014;22:296302.
41. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withaniasomnifera. Arabian Journal of Chemistry 2013;10:11931199.
42. Hayouni EA, Abedrabba M, BouixM, HamdiM. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian Quercus coccifera L. and Juniperus phoenica L. fruit extracts. Food Chemistry.2007;105:1126-1134.
43. Quispe-Candori S, Foglio MA, Rosa PT. MeirelesMA. Obtaining β-caryophyllene from Cordia verbenacea de Candolle by Super Critical fluid extraction. Journal of Supercritical Fluids.2008; 46:27–32
44. Achraf K, Boumediene M, Abdellah M,Saif G. European Journal of Scientific Research 2012; 80(3):311-321.
45. Abdullah S, Gobilik J, Chong KP. In vitro antimicrobial activity of *Cynodondactylon* (L.) pers. (bermudas) against selected pathogens. Developments in Sustainable Chemical and Bioprocess Technology.2013;16:227-237.
46. Takon I, Antai SP,OkonP. In-vitro evaluation of antibacterial activity of ethanolic and aqueous extracts of Ficus exasperata VAHL (Moraceae) leaves. Journal of the Formosan Medical Association. 2013;89(9):756-763.

47. Tim TP, Cushnie AJ. Antimicrobial Activity of Flavonoids. International Journal of Antimicrobial Agents.2005;26(5):343-356.
48. Omkar A, Jeeja T, Chhaya G. Evaluation of anti-inflammatory activity of Nyctanthesarbour-tristis and Onosma echioides. Pharmacognosy Magazine.2006;2(8):258- 260.
49. Obonga WO, Nnadi CO, Chima CC, Okafor SN,Omeje EO. In vitro antioxidant and In vivo Anti-inflammatory Potentials of MarantochloaLeucantha (Marantaceae) Extracts and Fractions. Dhaka University Journal of Pharmaceutical Sciences.2019;18(2):233-240.
50. Mohan M, Gulecha VS, Aurangabadkar VM, Balaraman R, Austin A, Thirugnanasampathan S. Analgesic and anti-inflammatory activity of a photosensitivity in humans. PLoS One.2008;7(3): 1-5.
51. Yankanchi SR, Koli SA. Anti-inflammatory and analgesic activity of mature leaves of methanol extract of Clerodendrum inerme L. (Gaertn). Journal of Pharmaceutical Science and Research 2010;2(11):782-785.
52. Pierre BK, Selim PH, Tatjana S. Study of polyphenol content and antioxidant capacity of Myrianthusarboreus (Cecropiaceae) root bark extracts. Antioxidant. 2015;4:419-426.
53. Okoli CO, AkahPA, Ezugworie U. Anti-Inflammatory Activity of Extracts of Root Bark of SecuridacalongipedunculataFres (Polygalaceae) African Journal of Traditional, Complementary and Alternative Medicines. 2005;2(3):54-63.
54. Rao BG, Ramadevi D. Evaluation of Anti-Inflammatory and Anti Arthritic Activity for Different Extracts of Aerial Parts of Cassia grandis linn. International Journal of Pharma Research and Health Sciences.2018;6(4):2723-2728.
55. Shorinwa OA, Enemuoh AO, Uche FI. Anti-inflammatory and analgesic activities of methanol extracts of stem bark of Anthocleistadjalonensis in wistar rats. Journal of Applied Pharmaceutical Science. 2015;5(11):117-120.
56. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology. 2006;5(4):357-61
57. Owoyele BV, Adebukola OM, Funmilayo AA. Anti-inflammatory activities of ethanolic extract of Carica papaya leaves. Inflammopharmacology. 2008;16:168-173
58. Uche FI, Ezugwu CO. Evaluation of antioxidant activities of some Nigerian medicinal plants. Asian Pacific Journal of Tropical Medicine. 2009;2(4):27-32.
59. Uche FI, Shorinwa OA, Okorie O, Mgbahurike AA, Chijioke-Nwauche I. Antinociceptive and anti-inflammatory activities of crude extracts of Ipomoea involucrata leaves in mice and rats. Asian Pacific Journal of Tropical Medicine. 2011;11:121-124.
60. Manthey JA, Grahmann K, Guthrie N. Biological properties of citrus flavonoids pertaining to cancer and inflammation. Current Medicinal Chemistry. 2001;8:135-153.
61. Franzotti EM., Santos CV, Rodrigues RH, Mourao MR, Antoniolli AR. Anti-inflammatory, analgesic and acute toxicity of Sidacardiafolia L. Journal of Ethnological pharmacology. 2002; 72:273-278.
62. Agada LO, Asije O, Okeri H. Anti-diarrhoeic Effects of Leaf Extract of Ipomoea involucrate. Nigerian Journal of Pharmaceutical Research.2005;4(1):11-14.
63. Sawadogo WR, Boly R, Lompo M, Some N, Lamien CE, Guissou IP, et al. Anti-inflammatory, Analgesic and Anti-pyretic activities of Dicliptera verticillata. International Journal of Pharmacology 2006;2(4):435-438.

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