The Journal of Phytopharmacology 2020; 9(1): 30-37
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2020; 9(1): 30-37
January- February
Received: 24-12-2019
Accepted: 27-02-2020

doi: 10.31254/phyto.2020.9106

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Secondary Metabolites, Pharmacognostic and Therapeutic Activities of the Rhizome Extract of *Curcuma longa* Grown in South-West, Nigeria

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ABSTRACT

Curcuma longa is an important plant with diverse medicinal properties. The aim of this study was to determine the chemical constituents and phenolic contents, ascorbic acid content, antioxidant, anti-arthritic and antibacterial potential of the rhizome extract of *C. longa* grown in South-West, Nigeria. GC-MS, UV-Visible Spectrophotometer, other established biochemical methods were used to investigate the rhizome extract. The GC-MS analysis revealed the presence of sixty (60) therapeutically active phytochemicals. The most abundant components were: nopol (23.0%), megastigma-3,7(*E*),9-triene (9.0%), curlone (7.0%) and (4E)-2-methyl-4-octenal (5.9%). The percentage of *α*-curcume, *α*-zingiberene and *β*-sesquiphellandrene were 0.9, 1.6 and 1.9%, respectively. The polyphenol, flavonoid and ascorbic acid values were 3,270.30 ±0.00 µgmg^{-1} GAE, 51.79±0.00 µgmg^{-1} QE and 51.48±0.03 µgmg^{-1} AAE, respectively. The rhizome extract of *C. longa* contained some phenolic compounds such as *o*-guaiacol (1.5%), *p*-vinylguaiacol (2.0%) and ferulic acid methyl ester (1.5%). The rhizome extract showed very low IC_{50} and therefore high AAI with Galvinoxyl (25.0 µgml^{-1} and 1.68) and DPPH (5.0 µgml^{-1} and 8.0). The total antioxidant capacity value was 1,173.08±0.00 µgmg^{-1} AAE. The rhizome extract also gave high anti-arthritic protein denaturation value between 86.82-69.32%. The extract was active against all the tested bacteria isolates showing high zones of inhibition (10.0-24.0 mm). The studies provide important information for efficient and effective uses of rhizome of *C. longa* for nutritional and therapeutic purposes.

Keywords: Curcuma longa, pharmacognostic, polyphenol, galvinoxyl, DPPH, antibacterial activities.

INTRODUCTION

Medicinal plants are considered as important sources of food and industrial raw materials [1, 2]. Many of them are also cultivated for their economic uses most especially in the pharmaceutical sectors. Several plants have been used locally to treat various diseases. Knowledge of phytochemistry is employed in the treatment of many diseases in human and animal [3-6]. Recently, there has been an increase in demand for natural products due to their therapeutic properties against oxidative stress and infectious diseases [7-9].

Curcuma longa Linn. also known as turmeric is a member of *Zingiberaceae* family. It is a herbaceous medicinal plant popularly cultivated as vegetable globally [10-17]. *C. longa* is a spice that has received much interest in both the medical and scientific fields. It was reported by Choudhary *et al.* [18]; Chun *et al.* [19]; Daily *et al.* [20]; Perkins *et al.* [21] that the plant is used as antispasmodic to smooth muscles, therefore reduces digestive and menstrual cramping. Moreover, it is also used in the regulation of male fertility [22]. It also helps in preventing melanoma and causes death of existing melanoma cells and reduces the risk of childhood leukaemia. Various parts of *C. longa* are natural detoxifiers for heart, liver, muscle, tissue and other organs and cells for proper functioning. It is also used to treat fever and ringworm in some part of Africa. Due to its natural cool and attractive yellow colour, the rhizome is used as a natural colouring agent as an approved food supplements and preservatives for human and animal [23-28]. It also helps in accelerating wound healing and aid the remodelling of damaged skin tissue. Therefore, makes skin fair, soft and smooth. The plant as a non-toxic agent is used locally to treat psoriasis and different inflammatory skin problems [29-31].

To the best of our knowledge, there is no enough information on the phytochemical, pharmacognostic, antioxidant and antibacterial potential of the rhizome extract of *C. longa* from South-West, Nigeria. Therefore, the present study was aimed at looking into the characterization of rhizome extract of *C. longa* and evaluating its modes of medicinal activities.

MATERIALS AND METHODS

Collection Sample and Preparation of Extract

The rhizomes of the plant were collected behind Saint Louis Grammar School, Kajola Street, Ikere, Ekiti State, Nigeria and it was identified as *Curcumin longa* Linn. Air dried and pulverised rhizomes were extracted using methanol. The mixture was then left at room temperature for at least 3 days, and then subjected to filtration. The percentage yield of the concentrated extract was calculated. The extract was then kept at 8 ºC in a refrigerator until used [32].

Compositional Analysis using Gas Chromatography–Mass Spectrometry

The GC-MS analysis of the rhizome extract of *C. longa* was carried out by means of a GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system equipped with an AOC-20i auto sampler. The separations were carried out using Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The other specifications for the analysis were set as previously reported by Ololade *et al.* [33].

Quantitative Screening of the Extract for Polyphenol

The amount of total polyphenol content in the rhizome extract of *C. longa* was determined with Folin-Ciocalteau reagent using the method previously described by Lay *et al* [34] with slight modification using gallic acid as a standard.

Quantitative Evaluation of Total Flavonoid

The evaluated amount of flavonoid in the rhizome extract was determined with aluminium chloride method as described by Cheng *et al* [35] using quercetin as a reference compound.

Quantitative analysis of Ascorbic Acid

The quantitative amount of ascorbic acid present in the extract was determined with 2,4-dinitrophenylhydrazine reagent as described by Benites *et al* [36].

Quantitative Determination of *In vitro* Antioxidant Activities

The Free Radical Scavenging antioxidant capacity of the rhizome extract of *C. longa* was tested using three different standard assays

(i) Evaluation of Galvinoxyl Radical Scavenging Assay

The antiradical potential of the extract was firstly accessed using Galvinoxyl reagent according to the procedure described by Kotora *et al.* [37] with slight modification. The percentage of the radical inhibition activity was calculated based on the following expression:

$$I\%_{\text{Galvinoxyl}} = \frac{A_{cont} - A_{ext}}{A_{cont}} X\ 100$$

A_{cont} and A_{ext} are the absorbance value for the control and extract solution, respectively.

(ii) Evaluation of 2,2′-diphenyl-1-picryl-hydrazyl (DPPH) Radical Scavenging Assay

The free radical scavenging and antioxidant potential of the extract was evaluated using DPPH according to the procedure described by Al-Reza *et al.* [38] with minor modification. The percentage of the radical inhibition activity was evaluated based on the following expression:

$$I\%_{DPPH^{\bullet}} = \frac{A_{blank} - A_{ext}}{A_{blank}} X\ 100$$

A_{cont} and A_{ext} are the absorbance value for the control and extract solution, respectively.

Estimation of Antioxidant Activity Index (AAI): The AAI was calculated as:

$$AAI = \frac{Galvinoxyl\ or\ DPPH^{\bullet}\ Initial\ Concentration}{\text{IC}_{50}}$$

AAI was classified as weak; when AAI < 0.5; moderate, when AAI ranged between 0.5-1.0; strong; when AAI ranged between 1.0-2.0; and very strong; when AAI > 2.0 [39].

(iii) Evaluation of Total Antioxidant Capacity

Evaluation of *in vitro* total antioxidant of the rhizome extract was carried out according to Kasangana *et al.* [40]. The total antioxidant capacity was expressed as equivalents of ascorbic acid.

Evaluation of Anti-Arthritic Activity by Fresh Hen Egg Albumin: *in vitro* anti-arthritic/anti-inflammatory activity of the extract was evaluated against protein denaturation method using fresh hen egg albumin according to the method previously used by Kiranmayi *et al.* [41] with slight modification. The extract concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

Determination of *In vitro* Bactericidal Potential

The antibacterial properties of the rhizome extract at different concentrations were evaluated by Agar-well diffusion method as previously described by Olawore and Ololade [42] with slight modification. Fourteen clinical isolates were used in all, out of which six were found to be Gram-positive bacteria namely: *Bacillus spp*, *Enterococcus faecalis*, *Micrococcus varians*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* while the remaining isolates were Gram-negative bacteria namely: *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens* and *Shigella dysenteriae*.

Evaluation of the Antibacterial Activity Index (AI): The AI of the extract was determined using the expression:

$$Antibacterial\ Index\ (AI) = \frac{Zone\ of\ Inhibition\ of\ Extract\ against\ each\ Bacteria}{Zone\ of\ the\ Reference\ Drug\ against\ each\ Bacteria}$$

Evaluation of the Relative Percentage Inhibition (RPI%): The RPI% of the test rhizome extract with respect to the positive control was calculated by using the method of Hepsibah and Jothi [43].

Results and Discussion

The Yield of Rhizome of *C. longa*

The percentage yield of the rhizome methanolic extract of *C. longa* was 5%.

Chemical Constituent of the Rhizome Extract of *C. longa*

The GC-MS analysis of the extract obtained from the rhizome led to the identification of sixty (60) medicinally active phytochemicals representing 98.9% of the methanolic extract obtained from the rhizome of *C. longa* grown in South-West, Nigeria. The compound, retention indices, percentage composition are given in Table 1.0, where the identified components are listed in order of their retention indices. The main components in the rhizome extract of *C. longa* were nopol (23.0%), megastigma-3,7(*E*),9-triene (9.0%), curlone (7.0%) and (4E)-2-methyl-4-octenal (5.9%). The percentage of the most common compounds in the *Curcuma* family such as *α*-curcume, *α*-zingiberene and *β*-sesquiphellandrene were 0.9, 1.6 and 1.9%, respectively. The rhizome extract of *C. longa* contained some phenolic compounds such

as such as *p*-vinylguaiacol (2.0%), *o*-guaiacol (1.5%) and ferulic acid methyl ester (1.5%). The previous studies on a similar species showed that the most abundant component in the rhizome and leaf essential oils of *C. leucorhiza* from India were germacrene (9.6–19.7%), curdione (19.1–19.5%), camphor (7.2–8.1%), 1,8-cineole (4.0–7.4%), curzerene (3.0–5.7%), linalool (5.2–5.4%), *neo*-curdione (2.8–4.6%) and isoborneol (2.0–3.8%) [44] while the major constituents in *C. aromatica* rhizome essential oil were: 8,9-dehydro-9- formyl-cycloisolongifolene (2.7–36.8%), germacrone (4.3–16.5%), *ar*-turmerone (2.5–17.7%), turmerone (2.6–18.4%), curdione (50.6%), camphor (18.8–32.3%), xanthorrhizol (26.3%), *ar*-curcumene (19.5%), di-*epi*-α-cedrene (16.5%), curcumol (35.8%), and 1,8-cineole (12.2%). The leaf essential oil contained camphor (24.0%–28.5%) and p-cymene (25.2%) as the main components [15]. Dosoky and Setzer [15]; Surwase *et al.* [45]; Amalraj *et al.* [46] reported that the components that are responsible for the aroma of *C. longa* are turmerone, arturmerone, and zingiberene. Davis *et al.*, [14]; Gupta *et al.* [47]; Hewlings and Kalman [48]; Rossino *et al.* [49] reported that curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5dione) and its derivatives which is the main active component of the rhizome of *C. longa* is a pleotrophic phytochemical with multiple therapeutic properties. Curcumin and its derivatives possess potential health benefits for human and animal to treat or inhibit the activities of numerous diseases such as diabetes, cancer, glaucoma, ophthalmic disorders, retinal microglial death, ischemia or reperfusion injury, cardiovascular disease etc. They also modulate a range of biochemical processes implicated in neurodegenerative disorders. Moreover, they help to purify and regulate the female reproductive system, the uterus and breast milk. Kumar *et al.* [50]; Thomford *et al.* [51] stated that curcumin and its analogues are also very important natural products for the good health of men due to the fact that they are associated and linked to the purification and building of semen.

Table 1: Chemical Composition of the Rhizome Extract of *C. longa*

Compound	Retention Index	Percentage Composition
6-oxa-bicyclo[3.1.0]hexan-3-one	782	0.1
γ-butyrolactone	825	0.1
5-methyl-4-hexen-3-one	838	0.5
tiglic acid	941	0.5
artemisia ketone	1042	1.0
(4E)-2-methyl-4-octenal	1048	5.9
isocitronellol	1074	1.0
o-guaiacol	1090	1.5
tert-amylbenzene	1107	1.5
2,3-dimethyl-2-phenylbutane	1142	1.5
7-methoxymethyl-2,7-dimethylcyclohepta-1,3,5-triene	1185	1.5
Spirobicyclo[3.3.0]octan-6-one-3-cyclopropane	1187	3.5
2-methyl-2-phenylpentane	1206	1.5
(2E)-2,7-dimethyl-2,6-octadien-1-ol	1228	0.5
megastigma-3,7(E),9-triene	1255	9.0
(+)-*trans*-chrysanthenyl acetate	1276	2.0
methylcaprinate	1282	0.5
nopol	1290	23.0
longipinene epoxide	1293	2.0
9-methyl-5-methylene-8-decen-2-one	1302	0.5
p-vinylguaiacol	1321	2.0
2-nitro-2-propenyl) cyclohexane	1328	0.8
2,6-dimethyl-6-nitro-2-hepten-4-one	1334	0.8
phenyl-3-methyl-2-butenoate	1344	1.0
methyl-2-methyl-2-phenylbutanoate	1373	0.5
methyl 10-methylundecanoate	1429	0.5
trans-α-bergamotene	1430	2.0
β-sesquiphellandrene	1446	1.9
α-zingiberene	1451	1.5
α-farnesene	1458	2.0
β-bisabolene	1500	0.5
α-curcumene	1524	0.9
D-nerolidol	1564	0.5
methyltridecanoate	1580	0.5
curlone	1582	7.0
1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1592	0.5
tumerone	1616	2.0
myristic acid	1769	0.02
dicumene	1795	0.5
2-methyl-2-phenylundecane	1803	0.5
methyl-14-methylpentadecanoate	1814	0.5
ferulic acid methyl ester	1855	1.5
methylhexadecanoate	1878	0.5
palmitic acid	1968	4.0
2-methyl-2-phenyltridecane	2001	0.5
2,2-dicyclohexylmalononitrile	2045	1.0
stearic acid, methyl ester	2077	0.05
methyl-(16E)-16-octadecenoate	2085	1.0
methyllinolelaidate	2093	1.0
methyl linoleate	2098	0.5
stearic acid	2167	0.02
oleic acid	2175	2.0
2-methyl-2-phenylpentadecane	2200	0.5
methyl arachisate	2276	0.5
arachidic acid	2366	0.02

methylheneicosanoate	2375	0.04
methyldocosanoate	2475	0.05
tributylacetylcitrate	2594	0.2
12-O-acetylingol-8-tiglate	3331	1.0
6Z-2,5,5,10-tetramethyl-undeca-2,6,9-trien-8-one	4229	0.5
Percentage Total		**98.9**

Evaluated Quantity of Total Phenolic Content

The quantitative assessment of the total polyphenol of the investigated rhizome methanolic extract of *C. longa* was 3,270.30±0.00 µgmg^{-1} gallic acid equivalent (GAE) (Table 2). Due to the presence of some medicinally active and low molecular weight phenolic compound such as *o*-guaiacol and *p*-vinylguaiacol etc in the rhizome extract the concentration of polyphenol gotten in the extract investigated in this study had a comparable polyphenol content with those reported in other species of *Curcuma* such as rhizome extracts of *C. amada*, *C. angusifolia*, *C. aromatica* and *C. zedoaria* from India with TPC values between 50-250 mgml^{-1} GAE [52]. Moreover, herbal tea of the dried rhizome powder of *C. zedoaria* purchased from local super market in Pakistan exhibited similar values of polyphenol between 5.90-9.74 mgg^{-1} GAE [53].

Evaluated Concentration of Flavonoid

The result of this study demonstrated that the methanolic extract of the rhizome *C. longa* possess high amount of flavonoid concentration with value of 51.79±0.00 µgmg^{-1} quercetin equivalent (QE) (Table 2), this value is similar to what was obtained for the related species such *C. amada* and *C. caesia* from India with TFC values 22.52 and 40.60 mgg^{-1} QE respectively. Likewise, herbal tea of the dried rhizome powder of *C. zedoaria* purchased from local super market in Pakistan had similar values of TFC values between 10.76- 17.12 mgg^{-1} QE [53]. Sahu and Saxena [54]; Chen *et al.* [55]; Cory *et al.* [56]; Lutz *et al.* [57] reported that polyphenols and their derivatives are known to be very active in helping the body build immunity and fight off unhealthy reactive oxygen species (ROS). Therefore, they help in reducing the risks and healing of different ailments and diseases such as arthritic ulcers, cancer, cardio-vascular diseases, proliferative inflammatory and gastro-intestinal problems.

Quantitative Concentration of Total Ascorbic Acid (Vitamin C)

The sample used in this study had high amount (51.48 µgmg^{-1} ±0.03 AAE) of ascorbic acid and its derivatives present in the rhizome of the plant (Table 2). This ascorbic acid value in the sample used in this study is comparable to the related species such *C. domestica* from Malaysia with the value of 0.41 mgg^{-1} [58]. Gallie [59]; Najeeb *et al.* [60] reported that ascorbic acid plays an important role in the body as antioxidant, wound healing, anti-ageing substance etc. It is important in the growth and maintenance of good and healthy cells, skin, cartilage, teeth, bone, and blood vessels.

Table 2: Polyphenol, Flavonoid and Ascorbic Acid Contents

Polyphenol	Flavonoid	Ascorbic Acid
3,270.30 ±0.00 µgmg^{-1} GAE	51.79±0.00 µgmg^{-1} QE	51.48±0.03 µgmg^{-1} AAE

Mean value ± Standard deviation of triplicate

Evaluation of Free Radical Scavenging and Antioxidant Activities

(i) Galvinoxyl Free Radical Scavenging and Antioxidant Activities

The Galvinoxyl percentage radical scavenging by the extract at various concentrations (1000. 500, 250, 125 and 62.5 µgml^{-1}) were 89.51, 85.80, 84.22, 83.33 and 82.29% respectively. The rhizome extract of *C. longa* with IC_{50} and AAI values of 25.00 µgml^{-1} and 1.68, respectively, the methanolic extract of *C. longa* had a similar Galvinoxyl free radical scavenging and antioxidant as ascorbic acid (the reference compound), which had IC_{50} and AAI values of 15.0 µgml^{-1} and 2.8 (Table 3). Galvinoxyl is a new method used as a probe in the study of radical reaction mechanisms. Galvinoxyl is a stable phenoxy radical which reacts with other radicals at a rate constant near the diffusion-controlled limit. Scavenging of radicals leads to decolourization and colorimetric determination is a way of estimating the efficiency of radical formation [61].

(ii) DPPH Free Radical Scavenging and Antioxidant Activities

At very low concentrations (1000, 750, 500, 250 and 125 µgml^{-1}), the range of the DPPH free radical inhibition by the extract were 74.10, 66.91, 60.43, 56.12 and 54.00%, respectively. The methanolic rhizome extract of *C. longa* had lower IC_{50} and higher AAI values of 5.0 µgml^{-1} and 8.0, respectively than ascorbic acid (reference drug), which had an IC_{50} and AAI values of 30.0 µgml^{-1} and 1.3 (Table 3). It was reported by Tamta *et al.* [62] that related *curcuma* species such as methanolic rhizome extract of *C. amada* from India had IC_{50} value of 18.98 µgml^{-1}. In a similar manner, Tariq *et al.* [53] reported that herbal tea of the rhizome dried powder of *Curcuma zedoaria* purchased from local super market in Pakistan had lower percentage of DPPH free radical scavenging values ranged between 36.57- 47.28%. Therefore, the rhizome extract of *C. longa* investigated in this study had higher radical scavenging and antioxidant potential than the reference compound and related species of curcuma. It is noteworthy, that the extract investigated in this study has been shown to be a good antioxidant agent even at very low concentrations. The results showed that the steric hindrance among adjacent bulky groups within a galvinoxyl molecule limited the extract to scavenge galvinoxyl radicals effectively unlike DPPH, while extracts showed a powerful capacity for scavenging free radicals in DPPH [61, 63, 64].

(iii) Evaluated Total Antioxidant Capacity

The quantitative estimate of the total antioxidant by phosphomolybdate method for the investigated rhizome extract of *C. longa* grown in Nigeria was found to be moderately high (1,173.08±0.00 µgmg^{-1} AAE) as shown in Table 3. Total antioxidant capacity of the extract investigated in this study is similar to those reported for the other species of *Curcuma* such as sequential methanolic extracts of the fresh and dry rhizomes of *C. caesia* from India with TAC of 1,198.67±79.66 and 1,542.67±5.51 mmol AAE/g extract, respectively [65]. The assay involves an electron transfer mechanism. It was reported by Bouayed and Bohn [66]; Lobo *et al.* [67]; Ahmed *et al.* [68] that natural antioxidants from vegetables are complex mixtures of different secondary metabolites of different activities, which may act synergistically to influence one another. They act by quenching free radicals from reactive oxygen species (ROS) or reactive nitrogen species (RNS) and by enhancing the deoxyribonucleic acid (DNA) enzyme repair systems through a posttranscriptional gene regulation of transcription factors. Studies by Rahman [69]; Huber *et al.* [70]; Wahlqvist [71]; Fiedor and Burda [72] showed that a diet rich in foods with high levels of natural antioxidants is associated with longevity and good health.

Table 3: Antioxidant Properties

Extract	IC_{50} µgml^{-1}	AAI	AAE µgmg^{-1}
GALV	25.0	1.7	
DPPH	5.0	8.0	
TAC	-	-	1173.08±0.00

Evaluated *In vitro* Anti-Arthritic Activity

The methanolic rhizome extract of the *C. longa* investigated in this study showed a remarkable anti-arthritic potential. Protein denaturation inhibitions at concentration between 62.5-1000 µgml^{-1} were observed as 69.32-86.82% with IC_{50} value of 20.00 µgml^{-1} (Figure 1). Aspirin (a standard anti-arthritic drug) showed the maximum inhibition of 90.00% at the concentration of 3.0 mgml^{-1}. From the previous studies by Dosoky and Setzer [15] the essential oils of *C. sichuanensis*, *C. nankunshanensis*, and *C. elata* exhibited lower percentages of inhibition of inflammatory activities of 68.43%, 55.23% and 54.64%, respectively, compared to the rhizome extract of *C. longa* investigated in this study which possessed higher percentages of anti-inflammatory potential. Hence, this study revealed that the methanolic rhizome extract of *C. longa* used in this study was capable of controlling the production of auto-antigens and also inhibits protein denaturation and membrane lysis in rheumatic disease [41]. This study showed that the extract exhibited strong inhibition of protein denaturation, therefore, could be potential source of natural anti-arthritic property which might be due to the presence of therapeutically active secondary metabolites such as polyphenols, terpenoids etc in the rhizome.

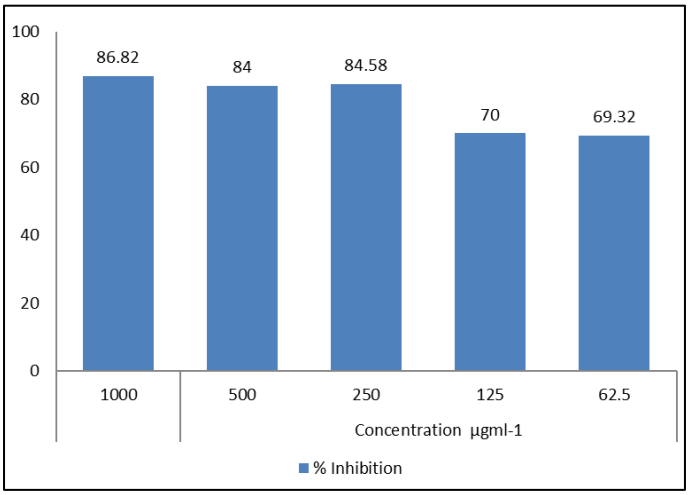

Figure 1: Egg Albumin Anti-Arthritic Activity of the Rhizome Extract

Evaluated *In vitro* Antibacterial activities

This study on the rhizome of *C. longa* established that its extract was active against all the tested multi-drug resistance Gram-positive and Gram-negative bacteria with the ZI, AI and RPI% values between 10.0-24.0 mm, 0.6-1.8 and 32.0-283.4, respectively (Table 4, Figure 2 and 3). The highest inhibition was observed with *M. varians* (24 mm). The extract of *C. longa* exhibited varying degrees of activities against the tested bacteria. It is noteworthy, equal or sometimes higher activities were observed at concentration of 1000-250 µgml^{-1} by the extract compared with Ofloxacin (standard antibiotic). Hence, the activity index, A.I≥1.0 against *E. coli*, *M. varians*, *P. aeruginosa*, *P. stuartii*, *S. aureus* and *S. marcescens*. No significant reduction in activities was observed as the extract concentrations were reduced gradually from 1000-250 µgml^{-1}. The rhizome extract investigated in this study had higher antibacterial activities compared to the extract of a related species such as essential oil from the rhizome of *C. aeruginosa* from Thailand which showed resistance or low inhibitions against *E. coli* (-), *S. aureus* (-), *B. subtilis* (9.0) and *E. faecalis* (11.0) [73]. Likewise, Devi *et al.* [44] reported that the leaf and rhizome essential oils of *C. leucorhiza* from India showed moderate inhibitions against *S. mutans*, *P. putida*, *B. subtilis*, *K. pneumoniae* between (7.0-16.0 mm). This is due to the presence of terpenoids and phenolic compounds in the extract. It was reported by Ololade *et al.* [33]; Fair and Tor [74]; Li and Webster [75]; Lillehoj *et al.* [76]; Sharma *et al.* [77] that in recent time, dangerous bacteria that are resistant to antibiotics have emerged with increasing frequency and damages done to human and animals health, therefore there is the need for the isolation of more effective and safer antibiotic phytochemicals from natural products.

Table 4: Table 4: Zones of Inhibition (mm) showing the Antibacterial Potential

	ZI of the Rhizome Extract			Ofloxacin
Conc. (µgml^{-1}) / Organisms	1000	500	250	5 µg
***Bacillus* spp (+)**	16	13	13	20
***E. coli* (-)**	21	18	15	12
***E. faecalis* (+)**	15	10	10	20
***K. pneumoniae* (-)**	18	16	15	22
***M. varians* (+)**	24	22	19	22
***P. aeruginosa* (-)**	18	18	17	18
***P. mirabilis* (-)**	12	12	12	18
***P. stuartii* (-)**	20	20	20	20
***S. agalactiae* (+)**	13	12	12	22
***S. aureus* (+)**	16	16	14	12
***S. dysenteriae* (-)**	12	12	12	15
***S. marcescens* (-)**	20	20	18	18
***S. typhimurium* (-)**	15	15	15	22
***S. saprophyticus* (+)**	13	12	12	22

Key note: Resistant (-), not sensitive (<8 mm), sensitive (9–14 mm), very sensitive, (15–19 mm) and ultrasensitive (>20 mm)

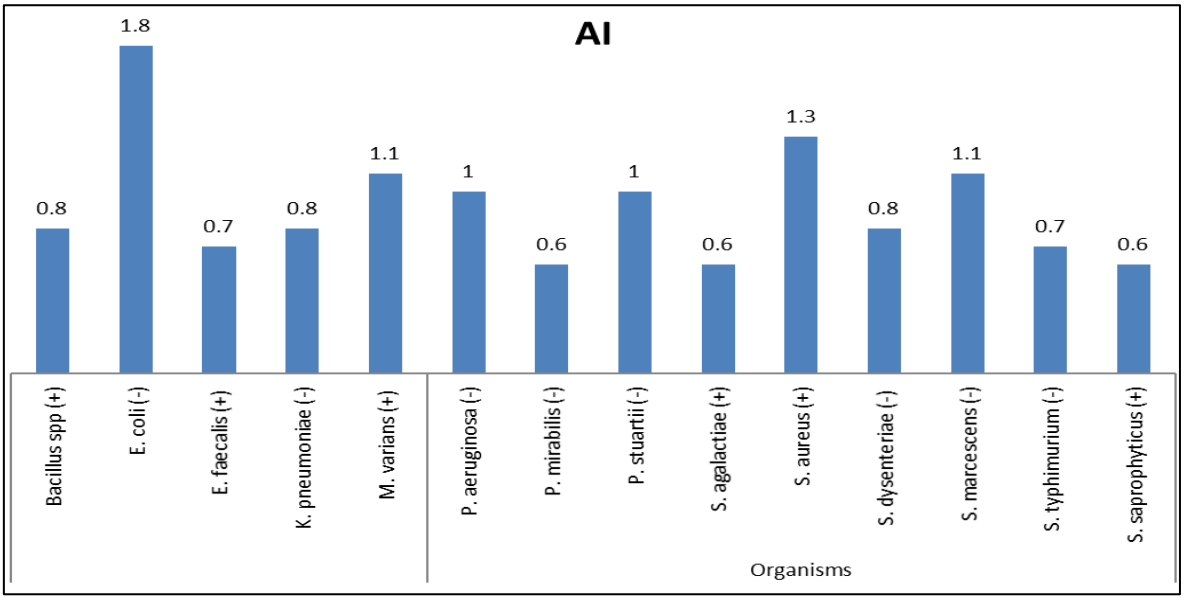

Figure 2: AI of the Extract against the Bacteria Isolates

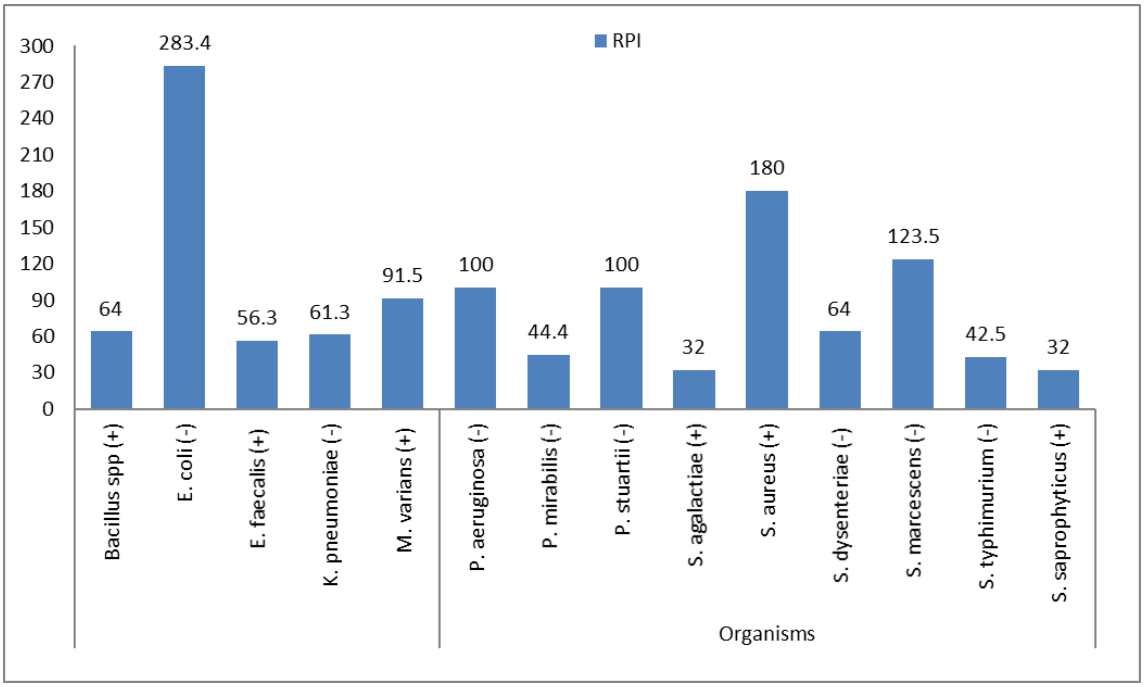

Figure 3: RPI of the Extract against the Bacteria Isolates

CONCLUSION

This study showed that the medicinal activities of the rhizomes of *C. longa* are due to some medicinally active phytochemicals present in it. Evidently, synergic effects of the terpenoids, polyphenol and other secondary metabolites in this part of the plant justified its traditional uses and pharmacological activities; this signifies that the extract of this plant may be useful as natural products to prevent oxidative stress and pathogenic related diseases. Therefore, the rhizome of the plant could be used for the development of cheap natural antioxidants, antibiotics, anti-inflammatory, anti-arthritic and other forms of drugs since it grows well and is easily accessible in our locality. The findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs. Further studies should be carried out on its clinical trial and on the other mechanisms that may still point out some of its medicinal properties which are not considered in this study.

Conflict of Interest: All the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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HOW TO CITE THIS ARTICLE

Ololade, Z.S., Abam, E.O., Anuoluwa, I.A., Abiona, O.O. Secondary Metabolites, Pharmacognostic and Therapeutic Activities of the Rhizome Extract of *Curcuma longa* Grown in South-West, Nigeria. J Phytopharmacol 2020; 9(1):30-37.