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EVALUATION OF THE BIOACTIVE COMPONENTS OF ETHANOL EXTRACT OF THE RHIZOME, CURCUMA LONGA USING ADVANCED SPECTROSCOPIC METHODS

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ABSTRACT

Medicinal plants have played a major role in the treatment of various ailments for thousands of years. They have proved to be a major source of remedies used for curing various disease conditions. *Curcuma longa* is a rhizomatous plant known to possess strong medicinal properties useful for the treatment of disease conditions such as inflammation, microbial infections and cancer. The study was carried out to determine the phytochemical components of *Curcuma longa* and to identify the different bio-active constituents that may account for its acclaimed medicinal benefits using advanced spectroscopic methods such as Furrier transmission infra-red spectrometer (FTIR) and hyphenated gas chromatography-mass spectrometer (GC-MS). Ethanol extract of the rhizome was obtained by extracting dry powdered samples of *Curcuma longa* rhizome with 70% ethanol and subsequently dried to obtain dried samples of the extract. Preliminary phytochemical screening of the sample revealed that tannins, terpenes, phenols and flavonoids were present in the extract. Functional groups such as hydroxyl, aromatic alkene and phenol were detected following FTIR analysis of the extract. These groups are however associated with good free radical scavenging activity and hence strong antioxidant property. The GC-MS analysis was equally complimentary. It revealed the presence of various useful bioactive compounds such as curlone, Ar-tumerone, hexadecanoic acid ethyl ester etc. Ar- tumerone and curlone have demonstrated good antioxidant, anti-inflammatory and anti-cancer activity while hexadecanoic acid ethyl ester has both antioxidant and hypocholestrolemic activity thus establishing the medicinal properties of *Curcuma longa* and its therapeutic benefits in the treatment of various disease conditions.

KEYWORDS: *Curcuma longa*, GC-MS, Phytochemical, Ar-tumerone, Curlone, hexadecanoic acid.

INTRODUCTION

The use of medicinal plants as remedies for a variety of ailments can be traced as far back as the stone age. The form in which they were often used has evolved over the years to include medicinal herbs, functional foods, teas and spices. The existence of thousands of species offers a wide range of options for the discovery and development of potent drugs with desired medicinal benefits. Their medicinal values have been linked to the presence of biologically active constituents found in their different parts. These biologically active constituents often referred to as phytochemicals or phytonutrients or bionutrients are primarily metabolites (secondary metabolites) produced by plants. Although they are not essential nutrients, meaning that plants do not rely primarily on them for their survival, they form an important part of their defense system. They function to help plants fight diseases as well as provide protection from damage and environmental hazards such as UV rays.[1] Some common phytonutrients found in plants include phenols and phenolic compounds, alkaloids, terpenoids, saponins, glycosides, tannins etc. Years of research and investigations has revealed that phenols and phenolic compounds are the most abundant phytonutrients found in plants.[1,2] Phenols and polyphenols are a group of large molecular weight and complex compounds which includes flavonoids, complex flavonoids, phenolic acids, colored anthocyanins and helps to protect plant from environmental and pathogenic.[3,4] They have also been found to possess anti-microbial, anti-oxidant, anti-inflammatory and anti-cancer properties when consumed by humans.[2,5,6] Studies have also shown their benefits in both the protection and management of cardiovascular and neurodegenerative diseases such as hypertension and diabetes. They have an abundant presence in vegetables e.g. pepper, onions, oil seeds e.g. rapeseed, olive seeds, canola, fruits e.g. grapefruit, apples, berries and beverages e.g. green tea, black tea, red wine and fruit juices.[4,7] Medicinal and aromatic plant species (MAPs) offer a variety of natural health remedies that have been broadly exploited as food flavoring, medicinal agents, preservatives and ornaments, as well as beauty and personal delight products, becoming natural alternatives that offer reliability, safety and sustainability. Amongst them, turmeric (*Curcuma longa* L., *Zingiberaceae*) is especially popular worldwide because of its culinary, cosmetic and medicinal uses.[8] *Curcuma longa* popularly known as turmeric is a rhizomatous plant having a characteristic orange-yellow color and largely cultivated in Asian countries like India, China, Japan as well as some tropical regions in Africa.[9] Its popularity results from its attractive culinary usage where it is used as a coloring and flavoring agent (it is the principal ingredient of the food condiment "curry" that is popularly used in Asian and most African cuisine due to its unique flavor, color and also its medicinal benefits.[10,11] Although *C. longa* is a perennial herbaceous plant, most of these interesting features and properties principally come from the rhizome, a horizontal underground stem from which the shoots and roots arise. It has distinctive organoleptic properties: a yellow/brown color externally, with a deep orange inner part, a special aromatic smell and a bitter, hot taste.[12] In traditional medicine especially in Chinese and Indian traditional medicine, *curcuma longa* is mostly used due to its anti-inflammatory, antioxidant, antimicrobial and anticancer properties.[13] it is also used for the treatment of digestive disorders, allergy, pain, fever, diarrhea, jaundice, wound healing, epilepsy, a variety of inflammatory conditions ranging from joint pains to skin inflammations.[14,15] *C. longa* rhizomes are a rich source of two major constituents with remarkable attributes: curcuminoids and essential oils.[16] Curcuminoids are responsible for the characteristic orange-yellow color of *C. longa*. They particularly refer to a group of three phenolic compounds: curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), desmethoxycurcumin and bis desmethoxycurcumin belonging to the diarylheptanoid family. Curcumin is the most abundant curcuminoid found in *C. longa*.[10,13] It is a yellow pigmented polyphenol has been identified as being responsible for most of its pharmacological properties such as anti-oxidant, anti-microbial.[17] as well as anti-cancer properties.[18] Its chemical structure is characterized by the presence of functional groups such as carbon-carbon double bonds, β-diketo functional group and phenyl groups to which a large number of hydroxyl and methoxy groups are attached which is believed to play a key role in its antioxidant abilities.[19] The antioxidant property of *C. longa* evidenced by its ability to scavenge free radicals including reactive oxygen and nitrogen species that are implicated in various cardiovascular and neurodegenerative diseases, is attributed to its hepatoprotective,[20] neuroprotective[11] and cardioprotective[10] properties.[21,22] As a potent inhibitor or COX-2 without any effects on COX-1, curcumin has also established itself as a potent anti-inflammatory agent for the treatment of arthritis offering an effective natural remedy with minimum side effects compared to conventional therapies.[23,24] Its chemoprotective property have also been well documented. It is known to inhibit the initiation, promotion and progression stages of carcinogenesis and has been reported to inhibit the growth of human colon cancer cells through the inhibition of cyclooxygenase-2 (COX-2) expression.[25,26] Also it inhibits the growth of ovarian carcinoma cells, breast cancer cells, prostate cancer and multiple myeloma in both invitro and invivo studies.[27,28,29,30] *Curcuma longa* L. rhizome is also known to contain essential oil which is a valuable product in the pharmaceutical industry due to its beneficial health effects. Essential oil of *C. longa* rhizome has been reported to comprise majorly of Zingiberene, Ar-tumerone, ar-curcumene, α- tumerone and curlone (β-tumerone).[31] These compounds have been reported to have strong anti-cancer, antioxidant and anti-inflammatory activity.[32,33]

MATERIALS AND METHODS

Preparation of Plant Material and Extraction

Samples of C. Longa rhizome was sourced locally from vendors in choba environ, Port Harcourt. Rivers state, Nigeria. It was identified and authenticated at the Department of pharmacognosy, University of Port Harcourt, Nigeria. Obtained samples were washed, cut into smaller pieces, dried in a shade, pulverized and extracted with a soxhlet apparatus using 70 % ethanol as the extraction solvent. The extract was concentrated and then dried at room temperature after which it was placed in a desiccator to remove any residual solvent.

Phytochemical analysis of plant extract

Phytochemical analysis was carried out to determine the presence of phyto compounds in the extract of the plant using established standard methods (Trease and Evans, 2009).

The extract was tested for carbohydrate by employing Molisch and Fehling tests. Also test for the presence of tannins and phlobatannins was performed using the ferric chloride test and hydrochloric acid test respectively. The frothing and emulsion tests was used to determine the presence of saponins while Shinoda test, ferric chloride test and Liebermann Burchard test was carried out to determine the presence of flavonoids, phenols and free anthraquinone respectively. Alkaloids were tested for using Dragendorff's and Mayer's reagent and the presence of terpenoids was also carried out.

FTIR Analysis

Analysis was carried out using agilent cary 630 FTIR spectrometer at a resolution of 4 cm^{-1}. Observed spectra was analyzed for characteristic peaks and patterns using Agilent resolution pro software.

GC-MS Analysis

Spectroscopic analysis of ethanol extract of *C. longa* was carried out on a gas chromatography-mass spectrometer instrument to determine the compounds present. Compounds were identified by comparing the spectrum of unknown phytochemical with those available in the NIST (National Institute of Standards and Technology) library where over 62,000 patterns have been documented. The comparism employed NIST version 2.0 library.

RESULTS

Table I: Phytochemical constituents of ethanol extract of *C. longa* rhizome.

S/N	Test	Ethanol extract
1	Alkaloids	-
2	Flavonoids	+++
3	Tannin	++
4	Phlobatannin	++
5	Saponin	-
6	Anthraquinone	-
7	Terpenoids	+++

FTIR Analysis

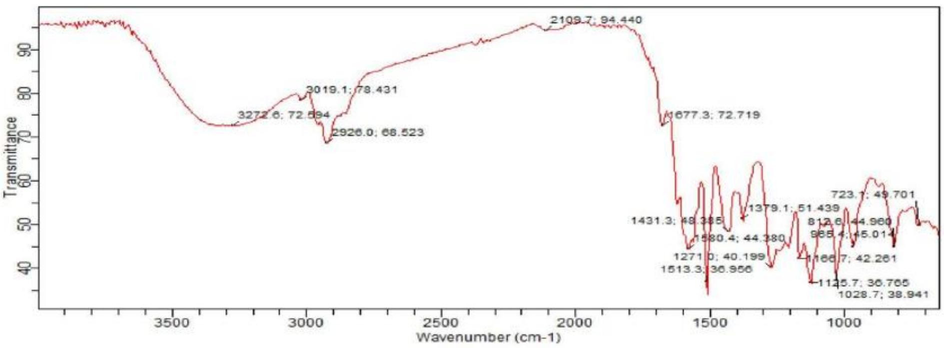
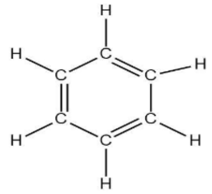
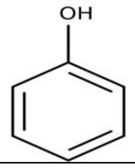


Fig. I: FTIR analysis of ethanol extract of *C. longa.*

Various peaks were observed from the spectrum above and the functional groups identified are summarized in table 2 below.

Table II: FTIR analysis showing functional groups present in the ethanol extract of *C. longa.*

S/N	Peak values (nm)	Intensity	Description	Functional groups	Inference
1	3272	72.594	Broad, Strong	Hydroxyl	O-H
2	3019	78.431	Medium	Alkene	C=C
3	2926	68.523	Sharp, Strong	Alkane	C-C

4	1677	72.719	Weak	Alkene (Aromatic, Saturated)	C=C
5	1580	44.38	Medium	Aromatic	
6	1513	36.956	Medium	Phenol	

GC-MS Analysis

The retention time and fragmentation patterns of the detected compounds were interpreted and compared with data on mass spectra library (NIST) which posted about 70% - 98% match quality.

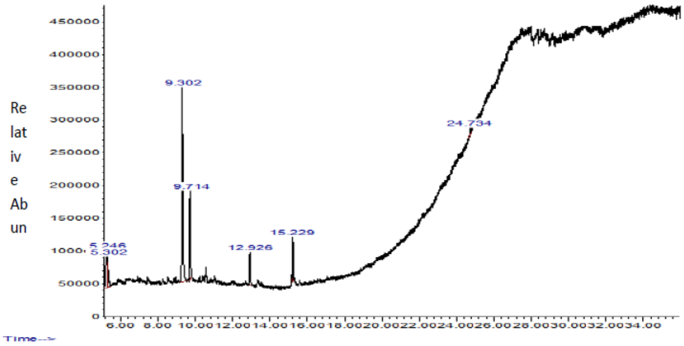


Fig. II: GC-MS chromatogram of ethanol extract of *C. longa*.

Table III: Chemical compounds present in ethanol extract of *C. longa* as identified by GC-MS analysis.

Peak #	Retention time (mins)	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	5.2456	Cis-1-methyl-2-(2-propenyl) cyclopropane	C_7H_{12}	96.1702	3.09
2	5.3025	9-oxabicyclo [6.1.0] nonane	$C_8H_{14}O$	126.1962	7.37
3	9.3025	Ar-tumerone	$C_{15}H_{20}O$	216.3187	48.13
4	9.7143	Curlone	$C_{15}H_{22}O$	218.3346	16.04
5	12.9255	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	6.02
6	15.2286	11-Hexadecanoic acid, ethyl ester	$C_{18}H_{34}O_2$	282.5	8.67
7	24.7338	Cyclohexanone-2-(2-propenyl)	$C_9H_{14}O$	138.2069	0.67

Table IV: Biological activity of some compounds present in ethanol extract of *C. longa*.

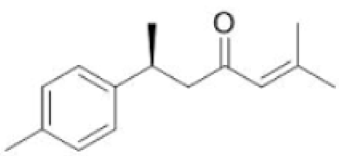
S/N	Chemical constituent	Class of Compound	Bioactivity	References
1	Curlone	Sesquiterpene	Antioxidant, anti-inflammatory	[33]
2	Hexadecanoic acid ethyl ester	Fatty acid	Antioxidant, hypocholesterolemic	[34]
3	11-Hexadecanoic acid ethyl ester	Ester	Antioxidant, hypercholestrolemic	[34]
4	Ar-tumerone	Sesquiterpene	Anti-cancer, antioxidant, anti-inflammatory	[32,33]

DISCUSSION

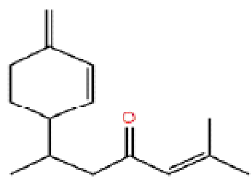
Medicinal plants are so called because of the presence of biologically active constituents that elicit various pharmacological responses in the body. They are often used for the treatments of various ailments such as bacterial infections, fungal infections, inflammation, pain, diabetes, hypertension, diarrhea, ulcer, malaria, typhoid fever, cancer etc. These medicinal properties are attributed to bioactive compounds present in plants and the existence of thousands of species is an enormous supply of raw materials and lead compounds for the development of modern medicines. *C. longa* has been used for thousands of years in ethno medicine for the treatment of various diseases such as diabetes, inflammation, pain, CVD's, bacterial infection and cancer.[21.22] The presence of bioactive compounds accounts for most of the therapeutic effects observed with *C. longa* when it is consumed. Phytochemical analysis of ethanol extract of *C. longa* identified carbohydrate, flavonoids, terpenes, tannins and phlobatannins as being present. The presence of flavonoids and terpenes supports the antioxidant property of *C. longa* while tannins and phlobatannins are indicated to have good anti-inflammatory and analgesic properties.

The FTIR results subsequently disclosed the presence of hydroxyl, aromatic alkenes, and phenol functional groups. The hydroxyl and phenolic groups have been heavily associated with free radical scavenging abilities hence strong antioxidant activity. Since free radicals are implicated in the pathogenesis of various diseases in the body such as CVD's and cancer, biologically active constituents with anti-oxidant activity have provided therapeutic benefits in the treatments of these diseases.

The Gas chromatography- mass spectroscopy analysis of the ethanol extract of *Curcuma longa* produced a total of seven chemical constituents. The main biologically active constituents present are ar-tumerone, curlone, Hexadecanoic acid ethyl ester and 11-Hexadecanoic acid ethyl ester. Other compounds present are Cis 1-methyl-2-(2-propenyl) cyclopropane, 9-oxabicyclo [6.1.0] nonane and cyclohexanone (See fig. II above). Ar-tumerone belongs to the class of compounds known as sesquiterpene. It is a volatile aromatic compound and commonly found in essential oil isolated from *Curcuma* plant species. It is biologically active and has anti-cancer, anti-inflammatory and antioxidant properties.[32,33] Curlone also known as β-tumerone is a major constituent of *C. longa* essential oil. It is a volatile compound and is classified as a sesquiterpene. Just like ar-tumerone, curlone has also demonstrated strong antioxidant, anti-inflammatory and anti-cancer activity.[33] Hexadecanoic acid ethyl ester and 11- hexadecanoic acid ethyl ester are both saturated fatty acids that have antioxidant and also hypocholesterolemic properties.[34] Cis 1-methyl-2-(2-propenyl, oxabicyclo [6.1.0] nonane**,** Cyclohexane-2(2-propenyl) have no known bioactive properties but are used in industries for manufacturing of non-pharmaceutical products.



Ar-tumerone



Curlone

Hexadecanoic acid ethyl ester

E-11-Hexadecanoic acid ethyl estsr

CONCLUSION

Curcuma longa contains a wide range of biologically active compounds with known medicinal benefits. Ethanol extract of *C. long* contains ar-tumerone, curlone, hexadecanoic acid ethyl ester and 11-hexadecanoic acid ethyl ester as its bioactive components. Other compounds present include Cis 1-methyl-2-(2-propenyl) cyclopropane, 9-oxabicyclo [6.1.0] nonane and cyclohexanone.

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