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Evaluation of Physicochemical and Antioxidant Potential of Fixed Oil from *Curcuma Longa* Linn.

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Abstract: *Curcuma longa* is used in traditional medicine in Nigeria for the treatment of diseases with etiologies linked to free radicals. This study was designed to investigate the physicochemical, phytochemical and antioxidant properties of the oil extracted from *Curcuma longa* rhizome. The plant was collected from its natural habitat and then identified. The sliced air-dried rhizome was powdered using grinding mill. The physicochemical parameters of the powdered rhizome were determined using standard procedures. The oil was extracted from the powdered rhizome using n-hexane. The solubility of the oil and the specific gravity were determined using standard methods. The oil was screened qualitatively for phytochemicals constituents and then analyzed for its chemical constituents using GC-MS. The acid value (AV), saponification value (SV), ester value (EV) and iodine value (IV) were determined. The acute toxicity was determined in mice using the Organization for Economic Co-operation and Development (OECD) method. The free radical scavenging activity was assayed using DPPH assay. The result of the physicochemical studies revealed moisture content (9.5 %) total ash (8.0 %) acid insoluble ash (3.5 %,), alcohol extractive value (4.8 %) and water extractive value (5.2 %). The percentage yield of the oil was 3.53 %. The oil was soluble in most of the solvents tested. The specific gravity of the oil was 0.98. The qualitative phytochemical screening of the oil revealed the presence of steroids and terpenoids. The GC-MS analysis of the oil showed the presence of tumerone, atlantone, caryophyllene, α-terpeniol, eugenol, curlone, octanoic acid-3-phenylpropyl ester and bisabolene. The AV, SV, EV and IV were 308.55

mg/g, 230.01 mgKOH/g, 78.54 mgKOH/g and 12.68 g/100g respectively. The acute oral toxicity study of the oil showed that oil was safe up to 5000 mg/kg per body weight. The oil exhibited free radical scavenging activity using DPPH. The results of this research showed that *Curcuma longa* oil can be used as a source of natural antioxidants.

INTRODUCTION

Fats and oils constitute a class of food called lipids and they have been reported to contain higher amounts of calories than carbohydrates (FAO, 2010). Furthermore, fats and oils are the most concentrated source of energy available to animal nutrition and form very vital part of the human diet (Alexandra *et al*., 2021). They are important ingredients in a variety of foods as they confer desirable characteristics on several foods (Raquel *et al*., 2014). Oils and fats are on high demand therefore, there is the need for their search from newer sources. Apart from their usage as food, they have been exploited for other purposes (Alexandra *et al*., 2021). The most recent increase in demand for oils and fats is seen in their utilization as biofuels and biodiesel (Morton *et al*., 2006; Ayhan *et al*., 2016). Biodiesel is a substitute to petroleum-derived fuels obtained from vegetable oils, animal fats, and waste cooking oils (Ayhan *et al*., 2016). In recent past, there is an increase interest in the use of biofuels over the world due to diminishing oil reserves, concerns about climate change from greenhouse gas emissions and the desire to promote domestic rural economies (Ozturk, 2014; Zulqarnain *et al*., 2021). Therefore, there is urgent need to search for oils from uncommon sources to meet up with these current demands. Vegetable oils have become the most discussed topic in the world due to the following factors: (i) increasing global demand for vegetable oils due to rising global wealth; (ii) rising awareness of health problems related to trans fatty acids (iii) increasing use of vegetable oils for biofuel feedstock supply as a substitute for fossil fuels and (iv) mounting international concerns over the environmental impacts of deforestation through palm logging and conversion of rainforest to cropland for soybean production (Morton *et al*., 2006).

Fats and oils are a group of macromolecules which play vital of biological roles in humans and other animals (Marcos *et al.,* 2021). Plants mainly store energy in their seeds in the form of lipids, and it is well known that plant triglycerides serve as energy sources for mammals (Marcos *et al.,* 2021). Nonessential oils also play important roles in plant protection serving as physical barriers against desiccation while also serving as signal molecules and plant hormones. In biochemical terms, a fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated (Marcos *et al.,* 2021). Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28 (Moss *et al*., 1997). Fatty acids are usually not found in organisms, but instead as three main classes of esters: triglycerides, phospholipids, and cholesterol esters. In any of these forms, fatty acids are both important dietary sources of fuel for animals and they are important structural components for cells.

Curcuma longa (turmeric) is a plant yielding rhizome which belongs to the Zingiberaceae family (Thomas, 2000). *Curcuma longa* has been reported to demonstrate several biological activities including anti-inflammatory effects due to curcumin (Akram *et al.,* 2010). The water and fat-soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity (Akram *et al*., 2010). Turmeric is known to have hepatoprotective anticarcinogenic (Kim *et al.,* 2005; Louay, 2014), antidiabetic and antimicrobial effects (Louay, 2014). Studies in animal models of Alzheimer's disease indicated a direct effect of curcumin in decreasing the amyloid pathology of Alzheimer's disease (Kukami and Dhir, 2010). *Curcuma longa* have been reported in Asian cuisines for both its flavor and colour. In Chinese and Ayurvedic medicine, it has been used for the treatment of inflammation, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic (Kim *et al*., 2005; Dewick, 2009).

The chemical constituents of turmeric rhizomes as revealed by GC-MS include volatiles and non-volatiles compounds. The aroma of turmeric is largely due to its volatile essential oil while the phenolic compounds, curcumin and its analogues account for its bright yellow colour (Champakam *et al.,* 2002). The components of turmeric named curcuminoids mainly consists of diferuloylmethane, demethoxycurcumin, and bismethoxycurcumin (Kojima *et al*., 1998). Curcumin is the important fraction which is responsible for the biological activities of turmeric. The active constituents of turmeric are the flavonoid curcumin (diferuloylmethane) and various volatile oils, including tumerone, atlantone, and zingiberone. Other constituents include sugars, proteins, and resins. The most studied active constituent is curcumin, which comprises 0.3–5.4 percent of raw turmeric (Khanna, 1999). *Curcuma longa* contains 1.08 % tannin. Tannin exerts antimicrobial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Prasad *et al.*, 2008). It is clear from the above that there is little or no information on the fixed oil component of *Curcuma longa* rhizome. Therefore, the aim of this research was to provide information on the chemical constituents of the fixed oil of *Curcuma longa*.

MATERIALS AND METHODS

Collection and identification

The plant was collected in July 2016 in Sokoto State-Nigeria. The plant was identified by Dr. H.E Mshelia at the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usman Danfodiyo University, Sokoto. Voucher specimen was prepared and assigned voucher number (PCG/UDUS/ZING/001). The specimen was deposited at the herbarium of the department for future reference.

Preparation of plant sample

The sliced rhizome was sun dried for 14 days to constant weight and then ground to powder with the aid of milling machine. The powder was stored in a polythene bag until required for use.

Physicochemical studies on the powder sample

The determination of moisture content, total ash, acid insoluble ash, water insoluble ash, alcohol-soluble extractive value and water-soluble extractive value were determined according to the methods described by Halilu *et al*. (2008).

Extraction of oil

The powder sample (1200 g) was weighed and transferred into the thimble and extracted with 1500 mL of hexane for four (4) hours. The extract obtained was transferred into an evaporating dish and then allowed to concentrate in an oven at 40 ^{o}C to produce the oil.

Qualitative phytochemical analysis

The qualitative phytochemical screening for the identification of oil using paper test, saponification test, Sudan III as well as testing for the presence of steroids in the oil using Lieberman-Burchard's and Salkowski's tests were carried out according to the methods described by Evans (2002), Halilu *et al*. (2008) and Sofowora (2008).

Chemical Analysis of the oil

The acid value, saponification value, iodine value and ester value were determined according to the procedure described by Halilu *et al*. (2017a)

Solubility testing

The solubility of the oil was tested in chloroform, water, petroleum ether, benzene, 70-30 chloroform/methanol mixture, 95 % alcohol, 100 % methanol. The solvents (2 mL) were transferred into separate test tubes and five (5) drops of the oil was added and then observed with the eyes for solubility (miscibility). Before the commencement of the experiment, heterogeneous mixture of water/oil consisting of 2 mL water and 5 drops of oil was prepared and served as the control (i.e., for visual comparison).

Determination of specific gravity

A measuring cylinder was cleansed thoroughly with NaOH and then allowed to dry. Furthermore, the measuring cylinder was rinsed with methylated spirit and then dried in an oven at 60 ^{o}C and was allowed to cool in a desiccator. The weight of the empty cylinder was measured using an electronic balance and recorded as W_1. Distilled water (2 mL) was transferred into the cylinder and the weight W_2 was recorded. The water was discarded and the test tube was dried. The oil (2 mL) was transferred to the cylinder and the weight W_3 was recorded and the specific gravity was determined.

GC-MS analysis

The analysis was carried out at Central Science Laboratory Usman Danfodiyo University, Sokoto. The analysis was carried out on Agilent Technologies Intuvo 9000 GC System and Agilent Technologies 5977B Mass Selective Detector (MSD) coupled with 4513A Automatic Liquid Sampler (ALS). The part number of the column used was Agilent 1909IS–483UI – INT capillary column with the specification HP – 5MS UI 30m, 0.25mm, 0.25µm, Intuvo. The carrier gas used was Helium at a flow rate of 1.2 mL/min. The injection volume was 1µl. The inlet temperature was maintained at 300^{o}C. The oven temperature was programmed initially at 50 ^{o}C for 5min at the rate of 5 mL/min. A total of 45 minutes run time was used. The MSD transfer line was maintained at a temperature of 250 ^{o}C. The source temperature was 230 ^{o}C and MS Quad at 150 ^{o}C. The ionization mode used was electron ionization at 70eV. Total Ion Count (TIC) was used for compound identification. The Spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST05 Reference Spectra Library.

Experimental animals

Mice (18 - 25 g) were obtained from the animal house of the Faculty of Pharmaceutical Sciences, Usman Danfodiyo University, Sokoto-Nigeria. The animals were acclimatized for 2 weeks before the commencement of the study. Standard animal feed and water were provided ad libitum. Housing conditions were maintained at 25 ^{o}C ± 2 ^{o}C at 12 h day/night cycles using an air conditioner. The study was approved by the Animal Research Ethics

Committee, Department of Pharmacology, Usman Danfodiyo University, Sokoto. The care and handling of the animals were done according to the established public health guidelines on Guide for Care and Use of Laboratory Animals (2011).

Acute toxicity studies

Oral acute toxicity study was carried out by 'Up-and- Down' method in mice according to Organization for Economic Development (OECD) guideline no. 425 (2008). A limit dose of the oil 5000 mg/kg was used for the study. Five mice of both sexes were used for the study. An animal was picked at a time, weighed and dosed with the equivalent volume of the oil. The oil was administered orally using gastric feeding tube and monitored according to OECD guidelines.

Qualitative Antioxidant assay using thin layer chromatography

This was done according to the method described by Halilu *et al*. (2017b). A TLC plate was cut into desired size and the oil was spotted using a capillary tube. This was allowed to develop in solvent system n-hexane/ethyl acetate (8:2). After development, the plate was allowed to dry and then sprayed with DPPH solution. The formation of yellow-colored spots against purple background indicates antioxidant activity (or free radical scavenging activity).

RESULTS

Physicochemical evaluation of the powder

The physicochemical evaluation of *Curcuma longa* powder revealed moisture content (9.5 %), total ash (8.0 %), acid insoluble ash (3.5 %), alcohol soluble extractive value (4.8 %) and water-soluble extractive value of 5.2 % (Table 1).

Oil Extraction, Qualitative Phytochemical Screening and Chemical Analysis of the Oil

The yield of the fixed oil was 3.53 %. The qualitative phytochemical test for the presence of steroids in the oil indicated positive result. The result of the chemical analysis indicated saponification value 230.01 mgKOH/g of oil, acid value 308.55 mgKOH/g of oil, Ester value 78.54 mgKOH/g of oil and iodine value 12.69 gI/100g of oil (Table 2).

Solubility studies and Specific gravity

The oil was soluble in chloroform, petroleum ether, benzene, 70/30 methanol/methanol, 95 % ethanol and methanol. The specific gravity of the oil was 0.98.

THIN LAYER CHROMATOGRAPHY AND QUALITATIVE

Antioxidant Activity of *Curcuma longa* oil

The result of the TLC analysis indicated the presence of several compounds as represented by the Rf values (Table 3). Some of the compounds showed antioxidant activity by scavenging free radicals as evidenced by the presence yellow or white spots on the TLC plates against the purple background. The result is shown on Plate 1.

GC-MS analysis of *Curcuma longa* oil

The GC-MS analysis of *Curcuma longa* oil revealed the presence of some volatile oil constituents such as α-terpeniol, eugenol, caryophyllene, tumerone, curlone, bisabolene and and atlantone (Table 4). Other constituent present include a fatty acid ester known as Octanoic acid-3-phenylpropyl ester (Table 4).

Acute toxicity studies

The LD_{50} was greater than 5000 mg/kg since no mortality and signs of toxicity were observed in the treated mice during the treatment period.

Table 1: Physicochemical evaluation of *Curcuma longa*

S/No.	Parameter	Quantity (%)
1	Moisture content	9.5
2	Total ash	8.0
3	Acid insoluble ash	3.5
4	Alcohol soluble extractive value	4.8
5	Water soluble extractive value	5.2

Table 2: Chemical analysis of oil

S/No.	Parameter	Amount
1	Saponification value	230.01 (mgKOH/g of oil)
2	Acid value	308.55 (mgKOH/g of oil)
3	Ester value	78.54 (mgKOH/g of oil)
4	Iodine value	12.69 (gI/100g of oil)

Table 3: TLC profile of the *Curcuma longa* oil

Spot	R_f value
H	0.9
G	0.8
F	0.7
E	0.6
D	0.5
C	0.4
B	0.3
A	0.2

Plate 1: Antioxidant activity

Table 4: GC-MS analysis of *Curcuma longa* oil

Component	Retention time (mins)	Quality (%)
α-terpeniol	6.65	35
Eugenol	7.79	98
Caryophyllene	8.32	97
Tumerone	10:35	98
Curlone	10:50	96
Bisabolene	11:20	98
Atlantone	11:52	93
Octanoic acid-3-phenylpropyl ester	13.09	27

DISCUSSION

Physicochemical evaluation is useful in establishing standards on identification, purity and quality of a plant sample intended to be used for drug (WHO, 2011; Emmanuel *et al*., 2020). The physicochemical analysis from this study revealed total ash of 8.0 % which is lower than the value reported by (Pawar *et al*., 2015). The acid insoluble ash was higher than the value obtained by (Pawar *et al*., 2015). This variation may be due to improper storage, where some little amount of silicaceous matter were deposited on the rhizome. This variation may also be attributed to the fraudulent intention of the herb collectors/ sellers to adulterate the sample in order to increase the quantity of the drug for higher gains and the same time reduce the quality of the drug (Harinarayan *et al*., 2011; Emmanuel *et al*., 2020).

The water-soluble extractive and alcohol soluble extractive values deviated from the previous studies (Pawar *et al.,* 2015) that reported the water soluble extractive value (15.16 %) and the alcohol soluble extractive value (16.10 %). These variation may due to adulteration (Emmanuel *et al*., 2020).

Phytochemical analysis of oil indicated the presence of steroids/triterpenes. Previous study on the powdered plant sample by (Shiyou *et al*., 2011) revealed the presence of steroids, steroids,tannins, glycosides, phenols, tannins, flavonoids and volatile oils. The saponification value of 230.01 mgKOH/g obtained from the study indicates the presence of higher molecular weight fatty acids. The acid value of 308.55 mgKOH/g indicates high proportion of fatty acids in the oil (William and Vida, 2015). Iodine value is a measure of the degree of unsaturation of fatty acids content of any fat or oil (as the value increases unsaturation increases) (Mowla *et al*., 1990). The iodine and ester values were not in concordance with the ones reported by (Meyer, 1987; Mowla *et al*., 1990; William and Vida, 2015). The deviation may be due to the variation of chemical compositions of the oils as it is a fact that, geographical location of plants significantly affect chemical composition while time of collection may affect the concentration of the chemical constituents (Yang *et al*., 2018; Alberto *et al*., 2020). The specific gravity indicates that the oil contains higher molecular weight fatty acids similar to those existing in olive oil (0.914) and cotton seed oil (0.917) (Meyer, 1987; Mowla *et al.,* 1990).

Solubility is one of the major parameters required to attain desired concentration of drug in a systemic circulation in order to elicit a pharmacological response (Muhammad *et al*., 2014). The solubility studies revealed that the oil is freely soluble in alcohol, petroleum ether, methanol, chloroform, benzene and chloroform/methanol (70/30). Therefore, it can be used in the formulation of pharmaceutical and cosmetic preparations.

The GC-MS analysis the presence of atlantone, curlone, bisabolane, tumerone. turmerone and bisabolene which according to their chemical nature are responsible for *Curcuma longa* aroma and smell (Li *et al*., 2011).

The qualitative screening of the antioxidant activity showed yellow spots against purple background on the TLC plates. This is preliminary evidence that the oil contains compounds that can exhibit antioxidant activity (Kojima *et al*., 1998; Halilu *et al*., 2017b). *Curcuma longa* oil exhibits antioxidant activity and can provide protection against oxidative stress due to free radical (Jaggi, 2012).

CONCLUSION

These results have shown that *Curcuma longa* oil exhibits antioxidant activity and can scavenge for free radicals. Furthermore, *Curcuma longa* oil can be used as source of natural antioxidants, used as food supplement and other applications in the pharmaceutical industry.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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