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# Comparative GC-MS determination of bioactive constituents of the methanolic extracts of *Curcuma longa* rhizome and *Spondias mombin* leaves

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**Abstract**
Over 80% of the world's population depends on herbal products for their primary healthcare requirements. The therapeutic potentials of many of these herbal products have been documented, however, more studies are needed to properly screen, identify and possibly characterize their various active ingredients. The present study attempts a comparative gas chromatography and mass spectrometry (GC-MS) determination of the bioactive constituents of the methanolic extracts of *Curcuma longa* rhizomes and *Spondias mombin* leaves. Results obtained indicated that although the extracts of both plants possess similar bioactive compounds: aromatic turmerone (ar-turmerone); turmerone and; cur lone the most abundant compounds were found to be aromatic turmerone for *Curcuma longa* and pentadecanoic acid for *Spondias mombin*. The possible biological effects of the identified compounds in both extracts are highlighted. Our findings provide rationale for the anecdotal use of both plants for treatment of similar medical conditions in our environment.

**Keywords:** GC-MS, herbal medicines, phytochemicals, biological properties

## Introduction

Over 80% of the world's population depends on herbal products for their primary healthcare requirements [1]. Consequently, several studies have explored the therapeutic potentials of most of these herbal products; however, more studies are needed to properly screen, identify and possibly characterize their active ingredients. It is also imperative to determine their modes of action, potential toxicity and possible interactions with orthodox medications [1-5]. Further, is the challenge of over dependence on ethobotanical products by many populations without scientific proof of beneficial claims by many trade-medical practitioners [1]. The proof of quality, effectiveness and safety of these products are frequently not considered before commercialization; thus overlooking the importance of safety of these herbal products [6]. As a panacea, the World Health Organization had stressed the need for in depth scientific research into herbal products [2]. In our environment, *Curcuma longa* (*C. longa*) and *Spondias mombin* (*S. mombin*) are typically known and used respectively as spice and herb [7, 8]. *C. longa* is a shrub that belongs to the ginger family Zingiberaceae. It grows to approximately a length of 1 meter with short stems and is mainly cultivated in Asian countries, principally India and Chinub-tropics [8]. It also serves as a food colouring agent much like curry (*Murraya koenigii*). *S. mombin,* also called hog plum, is of the family, Anacardiaceous; is widely cultivated in the tropical rain forest [7]. The plant has been reported to have a varied local application and potentially untapped values. The leaves, fruits and bark of *S. mombin* are said to be rich in antioxidants and other useful phytochemicals [9-11]. Gas chromatography (GC) is a reliable separation technique suitable for identification of small, gaseous or volatile molecules like alcohols, benzenes, aromatics, fatty acids, steroids and hormones [12]. GC is widely used for chemical analysis, drug screening and assessment resulting from environmental contamination. Mass spectrometry (MS) quantifies the mass-to-charge ratio of ions of analysts and is a plot of intensity as a function of mass-to-charge ratio [13, 14]. When combined Gas chromatography-Mass spectrometry (GC-MS) can separate complex mixtures, quantify analysts and determine trace levels of organic substances [15, 16]. Relying on the database of National Institute of Standards and Technology Chemistry Web Book [17] for different compounds, researchers can thus identify and quantify these potential compounds and constituents.

Considering several anecdotal reports of the potential beneficial effects of *C. longa* rhizomes and *S. mombin* leaves, this study attempts to determine the active ingredients in their methanolic extracts and possibly compare these outcomes using GC-MS technique. This we hope would attempt a phytochemical characterization of the methanolic extracts of both plants and a basis for their anecdotal use by traditional medicinal practitioners in our environment.

## Material and Methods

### Plant materials

Fresh rhizomes of *C. longa* were obtained from a Fruit and Garden Market in Port Harcourt Metropolis, Rivers State, Nigeria. Fresh leaves of *S. mombin* were also obtained from a private garden at University of Port Harcourt, Port Harcourt, Nigeria. Both plants were identified and authenticated by a plant taxonomist Dr. Ekeke, Chemezie of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Voucher samples were deposited in the herbarium and voucher numbers: UPH/P/165 for *C. longa* rhizome and UPH/P/166 for *S. mombin* leaves obtained.

### Extraction of Plant Samples

The rhizomes of *C. longa* and leaves of *S. mombin* were separately washed, air-dried and then ground to fine powder using a motorized electric grinder. About 1.4 kg and 0.1kg of the powdered forms of *C. longa* rhizome and *S. mombin* leaves respectively were separately soaked in 3.5 L and 0.5L respectively of methanol in covered jars. The content of the jars were macerated intermittently for proper mixing and each left to stand for about 72 hours. Each sample was then filtered and concentrated using a rotary evaporator at 45ºC. Separate semi-liquid extracts of the two plants were thus obtained, labeled and stored at a temperature below 4°C until ready for analysis. Analysis was done within 72 hours of extraction.

### Acute toxicity ($LD_{50}$) study

The acute toxicity of the methanolic extracts of *C. longa* rhizomes and *S. mombin* leaves were separately determined using the Lorke Method [18]. Consistent with previous reports, the $LD_{50}$ of each extract was found to be >2000mg/kg bw respectively [19, 20]. Ethical approval was obtained from our Institutional Ethical Committee and all experiments were conducted in accordance with guidelines for the care and use of laboratory animals **[21]**

### Method of GC-MS Analysis

GC-MS technique was used for the analyses of the plants extracts for the present study. The gas chromatography (GC) portion (Agilent technologies, United States of America, Model number 7890(B) was coupled to a mass spectrometer (MS) (Agilent technologies, United States of America, Model number 5975(B). The procedure adopted for the GC-MS analysis of both plants extracts for this study is as previously described [12, 22].

The analysis of result of GC-MS was done using the database of National Institute. Standard and Technology which comprise over 60,000 patterns [17]. The spectrum of the unknown constituent obtained was compared with the spectrum of the known components found in the NIST library. Attempt was made to establish names of compounds, molecular formula and molecular weights of each of the components identified in each extract evaluated.

## Results and Discussion

At least nine compounds were identified in the methanolic extract of the rhizomes of *C. longa* following GC-MS analysis: The names of compounds, retention times (RT), peak areas (in percentage), molecular formula and molecular weights of identified components are presented in Table 1. The predominant compounds, amongst others, in *C. longa* include aromatic turmerone (ar-turmerone) (25.07%), turmerone (21.20%) and curlone (16.81%). The GC-MS analysis of the methanolic extract of *S. mombin* leaves identified at least eight compounds. The names of compound, retention time (RT), peak area (in percentage), molecular formula and molecular weight of identified components in *S. mombin* extract are similarly presented in Table 2: The predominant components amongst others include pentadecanoic acid (14.75%), aromatic turmerone (ar-turmerone) (11.19%), turmerone (8.85%), n-hexadecanoic acid (8.33%), and curlone (7.56%). Figures 1 and 2 shows the GC-MS chromatographic representations of the components of the methanolic extracts of *C. longa* rhizomes and *S. mombin* leaves respectively. Interestingly, both plants extracts evaluated had three components in common. These are aromatic turmerone (ar-turmerone); turmerone and curlone. A number of the GC-MS peaks obtained were unidentifiable on account of lack of authentic samples and library of data of corresponding compounds. Nevertheless, this does not detract from the significance of the presented report. The possible biological effects of the identified constituents for both extracts are as indicated in Table 3.

Phytochemicals are described as a group of plant-based compounds and reports suggest that they may account for many of the beneficial effects attributed to fruits, vegetables and numerous plant-based products and diets such as tea, wine etc. [23, 24]. The determination of phytochemicals in beneficial and frequently consumed plant products is important [25]. Therefore, using gas chromatography and mass spectrometry (GC -MS) technique, the present study has attempted to identify and quantify compounds present in the methanolic extracts of *C. longa* rhizomes and *S. mombin* leaves.

The result presented in Tables 1 and 2 indicate that although, each plant extract possesses characteristic bioactive constituents; apparently, they both have three constituents' in common namely aromatic turmerone (ar-turmerone), turmerone and curlone. For instance, aromatic turmerone (ar-turmerone) 25.07% was found most abundant for *C. longa;* while pentadecanoic acid (14.75%) was found most abundant for *S. mombin* respectively. while the second and third most abundant compounds for *C. longa* were turmerone (21.20%) and curlone (16.81%); and for *S. mombin,* it was found to be aromatic turmerone (ar-turmerone) (11.19%) and turmerone (8.85%) respectively. However, curlone (7.56%) was found to be the fifth most abundant constituent for *S. mombin* extract. These findings suggest that the extracts of both plants would to a large extent possess similar biological effects as essentially outlined in Table 3. Thus, explaining the rationale for the anecdotal uses of the two plants for similar medical conditions: diabetes [26-28], hypertension [29, 30], lipid abnormalities [31, 32], antimicrobial [33], antioxidant [34], amongst others [35, 36]. Other compounds present are as indicated in Tables 1 and 2 for *C. longa* and *S. mombin* respectively. A comparison of the possible biological effects of the methanolic extracts of *C. longa* rhizomes and *S. mombin* leaves is as shown in Table 3.

**Table 1:** Chemical compounds identified in the methanolic extract of *C. longa* rhizomes.

| Name of compound | Retention time (RT) (Minutes) | Molecular formula | Molecular weight (g/mol) | Peak Area (%) |
|---|---|---|---|---|
| Aromatic turmerone (Ar-turmerone) | 16.986 | $C_{15}H_{20}O$ | 216 | 25.07 |
| Turmerone | 17.094 | $C_{15}H_{22}O$ | 218 | 21.20 |
| Curlone | 17.701 | $C_{15}H_{22}O$ | 218 | 16.81 |
| Benzenamine (aniline) | 16.471 | $C_6H_7N$ | 93 | 2.23 |
| 11-Octadecenoic acid (vaccenic acid) | 25.654 | $C_{18}H_{34}O_2$ | 282 | 2.22 |
| 1,3-Cyclohexadiene | 14.062 | $C_6H_8$ | 204 | 0.99 |
| 2-Butenoic acid | 19.818 | $C_4H_6O_2$ | 86 | 0.98 |
| 2-Methoxy-4-vinylphenol | 10.835 | $C_9H_{10}O_2$ | 150 | 0.94 |
| Methyl stearate | 26.221 | $C_{19}H_{38}O_2$ | 298 | 0.93 |

**Table 2:** Chemical compounds identified in the methanolic extract of *S. mombin* leaves.

| Name of compound | Retention time (RT) (Minutes) | Molecular formula | Molecular weight (g/mol) | Peak Area (%) |
|---|---|---|---|---|
| Pentadecanoic acid | 21.952 | $C_{15}H_{30}O_2$ | 243 | 14.75 |
| Aromatic turmerone (Ar- turmerone) | 16.957 | $C_{15}H_{20}O$ | 216 | 11.19 |
| Turmerone | 17.054 | $C_{15}H_{22}O$ | 218 | 8.85 |
| n-Hexadecanoic acid | 22.776 | $C_{16}H_{32}O_2$ | 257 | 8.33 |
| Curlone | 17.672 | $C_{15}H_{22}O$ | 218 | 7.56 |
| Oleic Acid | 26.501 | $C_{18}H_{34}O_2$ | 280 | 5.42 |
| Phytol | 25.900 | $C_{20}H_{40}O$ | 296 | 3.14 |
| 1-Eicosanol | 21.838 | $C_{20}H_{42}O$ | 299 | 0.61 |

**Table 3:** Possible biologic effects of identified compounds in the methanolic extracts of *C. longa* rhizomes and *S. mombin* leaves.

| Name of Compound | Extracts | Potential Biological Effects |
|---|---|---|
| 2-Methoxy-4-vinylphenol | *C. longa* | Naturally occurring phenolic compound used as a flavoring agent; possesses some anticancer and anti-inflammatory potentials [37, 38]. |
| 1,3-Cyclohexadiene | *C. longa* | Naturally occurring derivative of 1,3-cyclohexadiene is terpinene [39]. It has perfume and flavoring properties used mainly used to confer a pleasant odour to industrial fluids [40]. |
| Benzenamine (aniline) | *C. longa* | Predominantly used as a chemical intermediate for the dye, agricultural, polymer, and rubber industries; beyond trace level possess toxic effects on humans [41]. |
| 11-Octadecenoic acid (Vaccenic Acid) | *C. longa* | Naturally occurring trans-fatty acid found in the fat of ruminants and in dairy products such as milk, butter, and yogurt [42].<br>Predominant fatty acid comprising trans-fat in human milk [43].<br>Able to lower total cholesterol, LDL cholesterol and triglyceride levels in rats [43]. |
| 2-Butenoic acid (Crotonic acid) | *C. longa* | Beyond trace level possess toxic effects on humans [44]. |
| Methyl stearate | *C. longa* | Possess antibacterial property [45, 46]. |
| Aromatic turmerone (Ar-turmerone) | *C. longa* & *S. mombin* | It is known to possess as antioxidant, anti-inflammatory, and anti-nociceptive potentials; and stimulates the proliferation of peripheral-blood-mononuclear cells and elevates the synthesis of TNF-α, IL-2, and IFN-γ [47].<br>Promotes the maturation of dendritic cells and induces neural stem cell proliferation *in vitro* and *in vivo* [48, 49].<br>Both i*n vitro* (in human endothelial cells) and *in vivo* (in *Zebrafish* embryos and matrigel plug mouse model) studies reveal anti-angiogenic activities of aromatic-turmerone [48].<br>Promotes neural stem cell proliferation and differentiation in the brain [49]. |
| Turmerone | *C. longa* & *S. mombin* | Possesses toxic effects on mosquito larva [50]; immunostimulating properties [48]. |
| Curlone | *C. longa* & *S. mombin* | Natural anti-oxidant [51]. |
| 1-Eicosanol (Arachidyl alcohol) | *S. mombin* | Anticancer (against the breast cancer line MCF-7) and antimicrobial activities (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*, etc.) [52, 53] |
| Pentadecanoic acid | *S. mombin* | Found at the level of 1.2% in the milk fat from cows and reported to possess ability to regulate hormones, improve the immune system, boost metabolism [54-56]. |
| n-Hexadecanoic acid (Palmitic acid) | *S. mombin* | Anti-inflammatory properties; [57] Raised levels may increase the risk of developing cardiovascular disease [58]. |
| Phytol | *S. mombin* | Anticancer, Antinociceptive and Antioxidant Activities [12]. |
| Oleic Acid | *S. mombin* | It possesses beneficial effects on cancer, autoimmune and inflammatory diseases, besides its ability to facilitate wound healing [59]. |

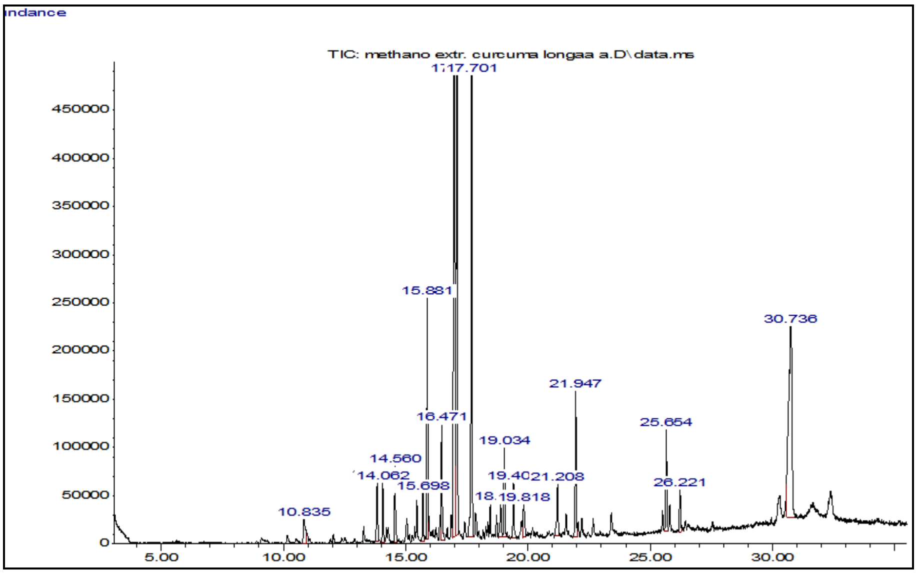


**Fig 1:** Chromatogram obtained from GC-MS screening of the methanolic extract of *C. longa* rhizomes.

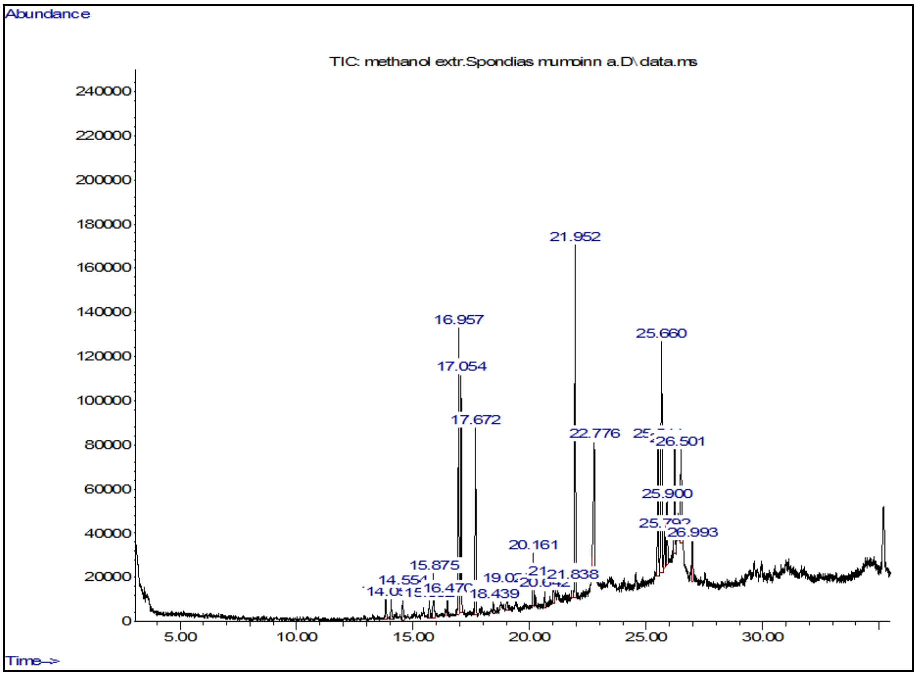


**Fig 2:** Chromatogram obtained from GC-MS screening of the methanolic extract of *S. mombin* leaves.

## Conclusion

In conclusion, the present study has been able to determine the possible chemical constituents of the methanolic extracts of *C. longa* rhizomes and *S. mombin* leaves using the GC-MS technique. A number of chemicals compounds identified were found in abundance in the extracts of both plants examined. However, the percentage proportion of these constituents differs in each extract. The possible biological effects of these identified chemicals were explored.

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