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Volatile constituents of five successive *Rhizophora racemosa* Leaf extracts G. Mey. (Rhizophoraceae)

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Abstract
The crude extract isolated from air dried leaves of Rhizophora racemose from ojo riverine using five different solvents. The leaves were analyzed for their constituents by GC/MS. Volatile constituents of Hexane extract contained fatty acids (91.58%) which is the predominant class in Hexane. monoterpene (3.10%) and diterpenes (3.72%) , 9-octadecenoic acid (49.94%), n-hexadecenoic acid (32.24%) and octadecanoic acid (6.61%) were the representative fatty acids. The GC-MS analysis also showed the presence of isoborneol (2.35%), and phytol (2.97%) as other compounds in amount greater than 1%. Main compounds observed in the dichloromethane extract consist majorly of hydrocarbon compounds which are tetratetracontane (18.30%), hexacosene (23.52%), 1-iodo- hexadecane (17.83%) and tricosane (12.75%). Ethyl acetate fraction on the hand consist majorly fatty acids (72.07%). A sizeable amount of diterpenes (7.86%), saturated hydrocarbons (7.56%) and monoterpene hydrocarbons (3.86%) were also present in the extract. The fatty acids content was represented in higher quantity n-hexadecanoic acid (39.75%) and oleic acid (25.13%). The diterpene, neophytadiene (7.86%) and a monoterpene, artemiseole (3.86%) were also present in a sizeable amount. Acetone extract of *R. racemose* contain four classes of compounds saturated hydrocarbons (74.99%), unsaturated hydrocarbons (2.08%), aliphatic alcohols (13.99%) and fatty acids (7.75%). The compounds identified from the acetone layer extract were mainly hydrocarbons. The significant compounds which belong to the hydrocarbon class include tetratetracontane (47.88%), cctacosane (12.25%), and 11-methyltricosane (7.18%). In addition, octadecanoic acid (7.75%), a fatty acid was also found in sizeable proportion. The alcohol contents were represented by 2-hexyl-1-decanol (11.2%) and 2-octyl-1-dodecanol (2.96%). The methanol extract phytochemical constituents of *Rizophora racemose* leaf extract comprised of aliphatic alcohols (11.19%) represented by 2-octyl-1-dodecanol (8.39%) and 2-hexyl-1-decanol (2.805). The fatty acid layer had a sizeable amount of octadecanoic acid (10.21%). The hydrocarbon contents which were detected in significant quantity (54.89% saturated and 7.04% unsaturated) included 9-octyl-docosane (35.29%), 13-undecylpentacosane (8.14%), hexatriacontane (7.66%) and 7-methyl-3,4-octadiene (4.71%).

Keywords: Rizophora racemose, phytochemical, GC/MS, volatile constituents

Introduction

Rhizophora racemosa G.F.W. Meyer is a species of mangrove plant of the family of Rhizophoraceae. It has a patchy distribution on the Pacific coast of Central and South America, occurs in places on the Atlantic coast of that continent, and has a more widespread range on the Atlantic coast of West Africa (Tomlinson, 2016)[18]. Out of the seven accepted members of the *Rhizophora* genus, *R. racemosa* is considered as underexplored since as far as literature could have established. Morphologically, R. racemosa is a tree reaching a height of up to 30 m developing stilt roots and elliptical leaves. This species has the potential to bloom 128 flowers on one axillary branch. Sepals of flowers are 8–10 mm long (Tomlinson, 2016)[18]. The Nigerian people traditionally used the leaves of *R. racemosa* to treat toothache and dysmenorrhea (Hubert O Dossou- Yovo, 2017)[12].

A study was conducted to determine the lethal dose (LD_{50}) of the methanolic leaf extract. Results showed that the LD50 of the extract was 1583.33 mg/kg which is considered safe for consumption (Be & Jc, 2014)[7]. In Benin, the roots of the mangrove plant, locally called Wéto, is used to manage malaria (Hubert O Dossou- Yovo, 2017)[12]. It is reported that the species *R. racemosa* from Australia contains primary and secondary metabolites, such as sugars and polysaccharides, aminoacids as well as polyphenols, triterpenes and tannins. The phytochemical profile (Sasidhar, 2020.). Results displayed that both methanolic leaf and bark extracts exhibited the highest radical scavenging, reducing potential and total antioxidant capacity (Sasidhar, 2020.). Phytochemical screening showed the isolation of secondary metabolites including apigenin, luteolin, vitexin, isovitexin, procyanidin B, quercetin and methoxy-trihydroxyflavone (Chiavaroli *et al*., 2020)[9].

A result revealed that the LD_{50} of the methanol extract of *R. racemosa* was 1583.33 mg/kg which is within safe level of consumption (Hubert O Dossou- Yovo, 2017)[12]. Extracts and compounds from the plant has shown antioxidant (Adi Prayitno & Rahmad Rahim, 2024; Chiavaroli *et al*., 2020; Ita & Eduok, 2022)[2, 9, 13], antibacterial activity *Staphylococcus aureus, Salmonella sp., Shigella sp., Vibrio cholerae and Vibrio parahaemolyticus* anong others)(Adi Prayitno & Rahmad Rahim, 2024; Chiavaroli *et al*., 2020; Ita & Eduok, 2022), antimycotic (Chiavaroli *et al*., 2020)[2, 9, 13], antifungal activity towards *Trichophyton mentagrophyte, Microsporum canis, Trichophyton rubrum and Epidermophyton floccosum (Bulan et al., 2022)*[8] as well as anti-diabetic (α-amylase, α-glucosidase), anti-tyrosinase and anti-cholinesterase (AChE, BChE) activities (Chiavaroli *et al*., 2020)[9]. The extracts of other species such as *R. mucronata R. apiculata, R. stylosa* have displayed varying degree of biological activities(Bulan *et al*., 2022)[8] and lot more (Ariole & Akinduyite, 2016.; Ukoima & Ikata, 2013)[20].

Materials and Methods

Collection and Identification of Plant materials

Plant materials (leaves) of *Rhizophora racemosa* was harvested from the wild growing along the coastal line, of ojo Local govt Area of Lagos State, Nigeria. (Voucher specimen bearing reference no. LUH:9043) identified by an expert taxonomist at the Botany Department Herbarium at the University of Lagos (UNILAG), Akoka, Lagos. The collected plant samples (leaves, was washed thoroughly under running tap water to free them from dust and other contaminants, air-dried for two weeks to remove the moisture content, pulverized, and the resultant powder was used for the extract preparation.

Preparation of Plant Materials for Extraction

The freshly collected leaf sample was rinsed and air-dried for 7 days. The dried samples pulverized into powder and stored in an amber bottle (Abuh *et al*., 2022)[1]. Five different solvents were used to extract the Leaves (Hexane, Dichloromethane, Ethyl acetate, Acetone and Methanol).

Sample Extraction

After Pulverizing the samples 300 grams of each sample was soaked in solvent sequentially using hexane, dichloromethane, ethyl acetate acetone and methanol, respectively. Filtration was done after 72 hours then followed by reconstitution of the final crude extracts(Abuh Omachoko Leonard *et al*., 2022)[1]. Each distillate isolated was preserved in a sealed sample tube and stored under refrigeration until analysis. Detailed data information was recorded.

Gas Chromatography / Gas Chromatography-Mass Spectrometry

Gas Chromatography / Gas Chromatography-Mass Spectrometry of the extracts was carried out and components identification was done by comparison of their retention indices with the authentic samples and matching of their mass spectra with the Wiley library mass spectra database as well as with published data(Babushok, 2015)[6].

GC-MS analysis

GC-MS analysis of the ethanolic extract of the various plants was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused a capillary column (30 × 0.25µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1). The injector temperature was maintained at 300 °C, the ion-source temperature was 250 °C, and the oven temperature was programmed from 100 °C (isothermal for 0.5 min), with an increase of 20 °C/min to 280°C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min.

Table 1: Compounds identified from the extracts of *R. racemosa*

S/N	Name	RI (Exp.)	RI (Lit.)	Percentage (%)				
				Dichloromethane	Ethyl acetate	Hexane	Acetone	Methanol
1	7-methyl-3,4-Octadiene	834	828	-	-	-	-	4.71
2	cis-Pinane	1018	1014	-	-	0.75	-	-
3	Isoborneol	1156	1160			2.35	-	-
4	2,8-dimethyl-1,8-Nonadiene	1200	1196	-	-	-	-	2.33
5	Artemiseole	1260	1252	-	3.86	-	-	-
6	n-Decanoic acid	1390	1387			0.66		
7	1-bromo-Tetradecane	1756	1758	1.65	-	-	-	-
8	Tetradecanoic acid	1770	1768	-	-	0.81	-	-
9	2-hexyl-1-Decanol	1772	1769	-	-	-	11.03	2.80
10	Palmitoleic acid	1955	1953	-	-	1.00	-	-
11	n-Hexadecanoic acid	1966	1964	4.00	39.75	32.24	-	
12	2-octyl-1-Dodecanol	1978	1978	-	-	-	2.96	8.39
13	Nonadecane	1900	1900	-	-	-	2.76	-
14	Eicosane	2000	2000	0.95	-	-	3.66	-
15	Oleic Acid	2100	2108	-	25.13	-	-	-
16	Heptadecanoic acid	2090	2086	-	-	0.66	-	-
17	(*E*)-3-Eicosene	2112	2100	1.38	-		-	
18	(*Z*)-9-Octadecenoic acid	2144	2146	-	-	49.94	-	-
19	Octadecanoic acid	2180	2172		7.19	6.61	7.75	10.21
20	Phytol	2119	2122	-	-	2.97	-	-
21	(*Z*)-11-Octadecenoic acid	2166	2161	-	-	0.32	-	-
22	1-Docosene	2196	2186	1.00	-	-	-	-
23	Docosane	2200	2200	1.23	-	-	-	-
24	1-iodo-Octadecane	2280	2272	1.50	-	-	-	-
25	Tricosene	2298	2298	2.73	-	-	-	-
26	Tricosane	2300	2300	12.75	-	-	-	-
27	11-Methyltricosane	2337	2330	-	-	-	7.18	-
28	1-iodo- Hexadecane	2360	2362	17.83				
29	Neophytadiene	2400	2395	-	7.86	0.75	-	-
30	Tetracosane	2406	2400	1.56	-	-	-	-

31	Pentacosane	2500	2500	1.04	-	-	-	-
32	Hexacosane	2600	2610	7.65	-	-	1.26	-
33	Hexacosene	2640	2620	23.52	-	-	-	-
34	Heptacosane	2700	2700	1.28	-	-	-	-
35	Octacosene	2798	2794	-	-	-	2.08	-
36	Octacosane	2810	2800	-	-	-	12.25	-
37	9-octyl-Docosane	2920	2900	-	-	-	-	35.29
38	Triacontane	3020	3000	-	-	-	-	3.80
39	Tetratetracontane	3400	3400	18.30	-	-	47.88	-
40	13-undecyl-Pentacosane	3530	3500	-	-	-	-	8.14
41	Hexatriacontane	3600	3600	-	7.56	-	-	7.66
Total				98.37	91.35	99.06	98.81	83.33
Monoterpene hydrocarbons (Sr. 2)				-	-	0.75	-	-
Oxygenated monoterpenes (Sr. 3,5)				-	3.86	2.35	-	-
Diterpenes (Sr. 20, 29)				-	7.86	3.72	-	-
Saturated hydrocarbons (Sr. 13,14,23,26,27, 30-41)				44.76	7.56	-	74.99	54.89
Unsaturated hydrocarbons (Sr. 1,4,17,22,25)				27.25	-	-	2.08	7.04
Aliphatic acids (Sr. 6)				-	-	0.66	-	-
Halogen alkanes (Sr. 7,24,28)				20.98	-	-	-	-
Aliphatic alcohols (Sr. 9,12)				-	-	-	13.99	11.19
Fatty acids (Sr. 8,10,11,15,16,18,19,21)				5.38	72.07	91.58	7.75	10.21

S/N = Elution order on Elite-5MS column; RI (Exp.) experimentally determined Retention indices; RI (Lit.) Literature retention indices; - not identified; Sr Serial number

The total contents of phytochemicals identified from the different extracts of the leaves of *R. racemosa* were 98.37%, 91.35%, 99.06%, 98.81% and 83.33% for the dichloromethane, ethyl acetate, hexane acetone and methanol, respectively (Table 1). Diverse chemical classes of compounds were identified from the analysis of the different extracts. From Table 1, saturated hydrocarbons (44.76%), unsaturated hydrocarbons (27.25%), halogen alkanes (20.98%) and fatty acids (5.38%) were the classes of compounds identified in the chloroform extract. The main compounds observed in the dichloromethane (CH_2Cl_2) extract consists mainly of hydrocarbon compounds which are tetratetracontane (18.30%), hexacosene (23.52%), 1-iodo- hexadecane (17.83%) and tricosane (12.75%). On the other hand, fatty acids (72.07%) constituted the bulk of the ethyl acetate extract and fractions of *R. racemosa*. A sizeable amount of diterpenes (7.86%), saturated hydrocarbons (7.56%) and monoterpene hydrocarbons (3.86%) were also present in the extract. The fatty acids content was represented in higher quantity n-hexadecanoic acid (39.75%) and oleic acid (25.13%). The diterpene, neophytadiene (7.86%) and a monoterpene, artemiseole (3.86%) were also present in a sizeable amount.

Although fatty acids (91.58%) were the predominant class of compound in the hexane, monoterpene (3.10%) and diterpenes (3.72%) could be seen prominent in the extract. In this extract, 9-octadecenoic acid (49.94%), n-hexadecanoic acid (32.24%) and octadecanoic acid (6.61%) were the representative fatty acids. The GC-MS analysis also showed the presence of isoborneol (2.35%), and phytol (2.97%) as other compounds occurring in amount greater than 1%. The four classes of compounds identified from the acetone extract of *R. racemosa* were saturated hydrocarbons (74.99%), unsaturated hydrocarbons (2.08%), aliphatic alcohols (13.99%) and fatty acids (7.75%). The compounds identified from the acetone layer extract were mainly hydrocarbons. The significant compounds which belong to the hydrocarbon class include tetratetracontane (47.88%), cctacosane (12.25%), and 11-methyltricosane (7.18%). In addition, octadecanoic acid (7.75%), a fatty acid was also found in sizeable proportion. The alcohol contents were represented by 2-hexyl-1-decanol (11.2%) and 2-octyl-1-dodecanol (2.96%). The phytochemical contents of the methanol extract of the leaves of *Rizophora racemosa* comprised of aliphatic alcohols (11.19%) represented by 2-octyl-1-dodecanol (8.39%) and 2-hexyl-1-decanol (2.805). The fatty acid layer had a sizeable amount of octadecanoic acid (10.21%). The hydrocarbon contents which were detected in significant quantity (54.89% saturated and 7.04% unsaturated) included 9-octyl-docosane (35.29%), 13-undecylpentacosane (8.14%), hexatriacontane (7.66%) and 7-methyl-3,4-octadiene (4.71%).

Results

The total contents of phytochemicals identified from the different extracts of the leaves of *R. racemosa* were 98.37%, 91.35%, 99.06%, 98.81% and 83.33% for the dichloromethane, ethyl acetate, hexane acetone and methanol, respectively (Table 1). Diverse chemical classes of compounds were identified from the analysis of the different extracts. From Table 1, saturated hydrocarbons (44.76%), unsaturated hydrocarbons (27.25%), halogen alkanes (20.98%) and fatty acids (5.38%) were the classes of compounds identified in the dichloromethane extract. The main compounds observed in the chloromethane ($CHCl_3$) extract consists mainly of hydrocarbon compounds which are tetratetracontane (18.30%), hexacosene (23.52%), 1-iodo- hexadecane (17.83%) and tricosane (12.75%). On the other hand, fatty acids (72.07%) constituted the bulk of the ethyl acetate extract and fractions of *R. racemosa*. A sizeable amount of diterpenes (7.86%), saturated hydrocarbons (7.56%) and monoterpene hydrocarbons (3.86%) were also present in the extract. The fatty acids content was represented in higher quantity n-hexadecanoic acid (39.75%) and oleic acid (25.13%). The diterpene, neophytadiene (7.86%) and a monoterpene, artemiseole (3.86%) were also present in a sizeable amount.

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Discussion

It has been observed that one of the biggest challenges of Africa as a continent is the obvious under exploitation and utilization of our forest resources, including mangrove trees. According to FAO, approximately 4 percent of Nigeria's rain forest disappear every day, which could have served as reservoirs for pharmaceutical/therapeutic precursors and industrial raw materials. There is a current global trend in the utilization of natural plant remedies thus creating enormous need for database on the properties and uses of medicinal plants.

Rhizophora species are medical plants of eastern and southeast Asia. Mangrove *Rhizophora* species which are the most common representatives are *Rhizophora racemosa, Rhizophora mucronata, Rhizophora mangle*, and *Rhizophora apiculata*. Mangrove plants are a group of trees and shrubs growing along seashores in tropical and subtropical areas (Tomlinson, 2016)[18]. *Rhizophora racemosa* G.F.W. Meyer is a species of mangrove plant of the family of Rhizophoraceae. It is found commonly in the Niger Delta region of Nigeria and along the West African coastline (Hubert O Dossou- Yovo, 2017)[12]. Out of the seven accepted members of the *Rhizophora* genus, only *R. racemosa* is considered as unexploited in the literature. Thus, our present study aims at shedding more light on this poorly understood plant.

A result revealed that the LD_{50} of the methanol extract of *R. racemosa* was 1583.33 mg/kg which is within safe level of consumption (Hubert O Dossou- Yovo, 2017)[12]. Extracts and compounds from the plant has shown antioxidant (Be & Jc, 2014; Chiavaroli *et al*., 2020; Ebube Samuel Izuogu *et al*., 2023a)[7, 9, 10], antibacterial activity *Staphylococcus aureus, Salmonella sp., Shigella sp., Vibrio cholerae and Vibrio parahaemolyticus* among others

(Chiavaroli *et al*., 2020; Ebube Samuel Izuogu *et al*., 2023a[9, 10]; Sasidhar, n.d.), antimycotic(Chiavaroli *et al*., 2020)[9], antifungal activity towards *Trichophyton mentagrophyte, Microsporum canis, Trichophyton rubrum and Epidermophyton floccosum* (Adi Prayitno & Rahmad Rahim, 2024)[2] as well as anti-diabetic (α-amylase, α-glucosidase), anti-tyrosinase and anti-cholinesterase (AChE, BChE) activities (Chiavaroli *et al*., 2020)[9]. The extracts of other species such as *R. mucronata R. apiculata, R. stylosa* have displayed varying degree of biological activities (Ita & Eduok, 2022)[13].

Several biologically active compounds have been isolated from various extracts of *R. racemosa.* These includes apigenin, luteolin, vitexin, isovitexin, procyanidin B, quercetin and methoxy-trihydroxyflavone which have shown antibacterial, antiviral, anti-diabetic, anti-cholinesterase and anti- tyrosinase inhibitory action (Chiavaroli *et al*., 2020)[9]. Several classes of compounds such as flavonoids, alkaloids, fatty acids etc were previously described from the plant(Bulan *et al*., 2022)[8]. Septicine, aureonitol, papuamine, di-iso-octylphtalate, 1-(2,4 dihydroxy-3,5-dimethylphenyl)-ethanone, cladosporin, tetrabenzofuran, dihydrophthalate, 9-octadecaenoic acid and eicosane were the compounds detected in the extracts from Nigerian grown sample (Ebube Samuel Izuogu *et al*., 2023b)[10]. Terpenoids were rarely described from the plant (Ukoima & Ikata, 2013)[20]. The methanolic *extract of R. racemosa* (Sasidhar, 2020.)*was reported to contained a higher quantity of 9, 12-octadecadienoic acid (Z, Z) methyl ester (39.896%).*

The phytochemical compounds of various extracts consisted of diverse structures. The main chemical constituents of the different extracts were known to be different from each other as could be seen in Table 1. n-Hexadecanoic acid was identified majorly in ethyl acetate (39.75%) and hexane (32.23%) extracts. While 2-hexyl-1-decanol (11.03%) and octacosane (12.25%) could be seen prominent only in the acetone extract, (*Z*)-9-octadecenoic acid (49.94%) was identified only from the hexane extract. Also, 1-iodo-hexadecane (17.83%), tricosane (12.75%) and hexacosene (23.52%) were described only from the dichloromethane extract. Of the studied five extracts, only the methanol extract contained 9-octyl-docosane (35.29%). Both the dichloromethane and acetone extracts had quantitative amounts of tetratetracontane at 18.30% and 47.88%, respectively.

Noteworthy of note was that variations were observed in the quantitative and qualitative compositions of the present study and previous ones described in the literature. It was noted that 9-octadecaenoic acid, identified in an earlier report (Ebube Samuel Izuogu *et al*., 2023b)[10] was also the main compound present in the hexane extract (Table 1). Although eicosane was present in a significant amount in the present study, however, 9, 12-octadecadienoic acid (*Z, Z*) methyl ester, which was identified earlier in another study on methanolic extract (Sasidhar, 2020.) was conspicuously absent in this study. Moreover, several compounds of the present study such as n-hexadecanoic acid, oleic acid, 2-hexyl-1-decanol, hexacosene 9-octyl-docosane and tetratetracontane, among others, were not identified in previous studies to be major constituents of *R. racemosa* extracts. These variations may be due to the nature and age of plant parts used, method of extraction, environmental conditions at the place of collection among others (Kannappan *et al*., 2021)[14].

The phytochemical compounds of some other *Rhizophora* species growing in other parts of the world have been described. The analysis of chemical constituents of *R. apiculata* was found to be rich in 2-(2-ethoxyethoxy)

ethanol (26.45%) and kaur-16-ene (3.37%), while benzophenone (16.09%) and 2-(2-ethoxyethoxy) ethanol (7.82%) were the predominant constituents in *R. mucronata* (1). The main phytoconstituent reported in *R. (Udeozo et al., 2408)* were tetramethyl-6,7,8,8a-tetrahydro-5H-naphthalene-1-one (38.63%), squalene (31.19%), α-amyrin, (7.07%) and β-amyrin (8.75%). From these analyses of *R. apiculata* n-hexane extract (Ariole & Akinduyite, n.d.) , the major constituents were identified as 2,6-dimethoxyphenol (28.80 %), guaiacol (18.14 %), p-cresol (7.82 %) and 1,2,4-trimethoxybenzene (7.00 %). The floral scent of *R. stylosa* from Japan was composed of several phenylpropanoids, mainly 1,2-dimethoxybenzene and eugenol, in addition to other class of volatiles, 2,3-butanediol and linalool (Auni *et al*., 2013)[4]. These compounds (except 2,3-butanediol) are well known as floral scent volatiles of other plants (Auni *et al*., 2013)[4]. The GC-MS analysis of *R. apiculata* leaf was found to be rich 2-(2-ethoxyethoxy) ethanol (26.45%) and kaur-16-ene (3.37%), while 2-(ethoxyethoxy) ethanol (11.08%) and butyl cyclohexyl ester 1,2-benzenedicarboxylic acid (3.48%) were the main components in the flower. Octadecamethyl cyclononasiloxane (5.24%), kaurene (3.39%) and 1,2,3,4-tetramethoxy-5-(2-propenyl)-benzene (3.26%) were the predominant constituents in the stem(-Wosu, n.d.). The major peaks found in some *Rhizophora* species were 8-pentadecane, 1, 2, 5-trimethylphyrrole, di-(2-ethylhexyl) phthalate, diethyl phthalate, epoxyhexobarbital and cyclooctacosane(Tjk, 2022)[17].

It could be seen that the chemical constituents of the volatile extracts of *R. racemosa* were quite different from other species described in the literature. It has been postulated that different plant species stored different phytochemicals. These phytochemicals are responsible for the variations in the biological and pharmacological activities of the species (Azuma *et al*., 2002; Sallehudin *et al*., 2023)[5, 15].

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