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Phytochemicals, chemical composition and antioxidants profile of the crude extracts and essential oil of *Mitracarpus scaber* (Goga masu)

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Abstract
Nowadays, more and more interests are focused on compounds that inhibit reactions promoted by free radicals. The study shows the phytochemicals constituents, chemical compositions and antioxidant activity of crude extracts and essential oils from *Mitracarpus scaber*. Phytochemical screening of crude methanolic extract of plant revealed the presence of saponins, tannins, flavonoids, essential oil and glycosides. Bioactive compounds present in essential oil *Mitracarpus scaber* of were identified using gas chromatography-mass spectrometry (GC-MS). Twenty-eight compounds were identified including phenolic and alcoholic compounds, as well as fatty acids. The components with highest concentration were azetidine, 1-benzyl-3, 3-dimethyl-2-phenyl- (9.60%) and 7-Heneicosane (4.61%). The antioxidant activities (DPPH, FRAP and hydrogen peroxide) were measured via spectrophotometry. The DPPH, H_2O_2 and FRP activities increased with increasing crude extract and essential oil content. The above extract and the essential oil have shown the significant scavenging activities when compared with standard ascorbic acid. However, the essential oil is considered to be better radical scavengers than methanolic extract. Our results suggest that essential oil from *Mitracarpus scaber* are a good source of natural products with antioxidant properties for potential therapeutic, nutraceutical and functional food applications.

Keywords: Scavenging activities, free radical, DPPH, FRAP, GC-MS.

1. Introduction
In the last century, a tremendous progress in medicinal plants research has been observed. In fact, the world is concerned towards the use of traditional medicine which has created a great interest towards plant and plant extracts. Essential oils are among the most interesting components of the plant extracts consisting mostly of monoterpenoid or sesquiterpenoids. They are used as therapeutic agents in ethno, conventional, and complementary alternative medicines particularly as analgesic, anti-oxidants, anti-inflammatory, antispasmodic, local anaesthetic, anthelmintic, antipruritic, and antiseptic as well as many other therapeutic uses and disease control [1]. Several lines of studies have also reported that essential oils are used broadly in medicine and cosmeceutical and pharmaceutical industries and as flavouring agents and preservatives in food industry and design [1] (Yahaya *et al*., 2018). Due to the increasing demand for natural ingredients, the use of essential oils for food preservation appears as a viable and healthy alternative to unpopular synthetic antioxidants. Therefore, this work was carried out to evaluate the chemical composition of *Mitracarpus scaber* as well as their phytochemicals and antioxidant activity. Foremost, the extractions of essential were optimized using turbo hydrodistillation, then they were analyzed for their possible antioxidant activities by three methods, namely the H_2O_2 assay, Ferric Reducing Antioxidant Power and the DPPH assay for their radical scavenging activity. The objective of this study was to determine the possibility of using the Essential Oils as food additives for the food industry in order to satisfy the consumer demands by reducing the use of synthetic antioxidants.

2. Materials and Methods
2.1 Materials and Reagents
Methanol, Soxhlet apparatus, rotary evaporator, filter papers, freeze dryer, n-hexane and ethyl acetate, DPPH, H_2O_2, etc.

2.2 Sample collection and Preparation
Fresh sample of the leaves of *mitracarpus scaber* was collected in Yola North Local Government Area of Adamawa State. Fresh leaves of the plant were dried under shade and grind in a sterilized iron motor and pestle to reduce the particle size, the powdered sample will then be stored in a clean container before used.

2.3 Methods
2.3.1 Phytochemical screening
Phytochemical screening of all the evaporated solvent extracts was conducted in accordance with the standard procedure [2]. Test for saponins, tannins, flavonoids, essential oils, glycoside, alkaloids, phenols and resin were carried out in all the fraction

2.4 Extraction of essential oils
500g of the pulverized from each of the fresh samples was subjected to steam distillation in a modified steam distiller (as modified by Runde *et al*., [3] according the British pharmacopoeia (BP) method. The time taken for the isolation of the oil was 4 hours [4].

2.5 Analysis on Essential Oil
2.5.1 Gas chromatography-mass spectrometry (GC-MS)
GC-MS analysis was performed on a J and W Scientific gas chromatography directly coupled to the mass spectrometer system (model GC Agilent technologies 7890A, Agilent technologies MSD 5975C), 5 % phenyl methyl silox: 469.56 509 packed capillary column (30M x 250µm) was used.

2.5.2 Identification of Compounds
Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST). The mass spectrum of the unknown components was compared with the spectrum of the known components that stored in the NIST library.

2.6 Determination of Oxidant Activity of Essential Oils
3.6.1 DPPH free radical scavenging assay
The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant activity of the various essential oils. This assay measures the free radical scavenging strength of the tested essential oil. The essential oil was dissolved in methanol, and various concentrations (2, 6, 12, 24, and 50µL/mL) was used. The assay mixture contained in a total volume of 1 mL, 500 µL of the oil, 125 µL prepared DPPH (1mM in methanol), and 375 µL solvent (methanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation below:
% or radical scavenging = [(Abs control – Abs Sample) ÷ Abs control] x 100 [3].

3.6.2 Hydrogen peroxide radical scavenging activity
This method proposed by Yahaya *et al*. [1] was adapted. 100 µl each plants sample (WCF, CNL, CCL and CRL) [25- 250 µg/ml in 50 mM phosphate buffer, pH, 7.4] was mixed with 300 µl phosphate buffer (50 mM, pH,7.4) and 600 µl H2O2 solution (2 mM in 50 mM phosphate buffer) and vortexed. Then after 10 minutes the absorbance was recorded at 230 nm using UV- Visible spectrophotometer (Systronics, AU2701). The phosphate buffer (50 mM, pH, 7.4) and vitamin C was used as blank and standard respectively. The hydrogen peroxide scavenging activity was determined using the formula [1].

3.6.3 Ferric Reducing Power Assay
This method proposed by Yahaya *et al*. [1] was adapted. 1 ml each plant extract (WCF, CNL, CCL and CRL) [100-500 µg/ml] was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml K3Fe (CN)6 (1 %) and incubated at 50 °C for 20 minutes. Then 2.5 ml trichloroacetic acid (10 %) was added to the solution and centrifuged at 3000 rpm for 10 minutes. The 2.5 ml of this solutions was mixed with 2.5 ml distilled water and 0.5 ml FeCl3. Then absorbance was measured at 700 nm using UV Visible spectrophotometer (Systronics, AU-2701). The vitamin C was used standard and reducing power was determined as vitamin C equivalent per 100 gm of dry sample [1].

3. Results and Discussions
3.1 Basic Phytochemical Screening of *Mitracarpus scaber*
From table 1, the result of phytochemical screening of methanolic extract of the leaves of *Mitracarpus scaber* have essential oils. All the plant extract contains saponins, tannin, flavonoid and glycoside. Alkaloids was not detected in *Mitracarpus scaber*. Both phenol and resin were absent in *Mitracarpus scaber. Mitracarpus scaber* contain the basic phytochemical accepts alkaloids, phenol and resin.
In another work by Shinkafi *et al.*, [5], the reverse was the case when he analysed the qualitative analysis of *Mitracarpus scaber* leaves, his result revealed abundance of saponin and flavoniod and presence of tannin, alkaloid, glycosides and steroids.
Mitracarpus scaber in this study reveals abundance of glycoside, steroids, tannin and presence of flavonoid, saponin and alkaloid while phenol was absent. This result is also in agreement with study by Chilaka *et al.*, [6] and Ouadja *et al.*, [7] in their study phytochemical analysis of Mitracarpus scaber leaves reveals presence of alkaloids, tannin, cardiac glycosides and saponin.

Table 1: Qualitative phytochemical analysis

Phytochemicals	*Mitracarpus Scaber*
Saponins	+
Tannins	++
Flavonoid	+
Alkaloid	ND
Essential oils	++
Glycosides	+++
Phenols	ND
Resins	ND

Note: + = Trace of the compound, ++ = Moderate constituent, +++ = Large Constituent and ND = Not detected

3.2 Percentage yield of essential oil from *Mitracarpus scaber*
1000g of fresh plant material was subjected to steam distillation method for extraction of essential oil. The results obtained shows that *Mitracarpus scaber* leaves has the yield of 0.54%.

3.3 Chemical Composition of Essential Oil from *Mitracarpus scaber*
In table 2 presented the chemical components of

Mitracarpus Scaber essential oil. The GC-MS analysis identifies 28 components with azetidine, 1-benzyl-3,3-dimethyl-2-phenyl- and 7-Heneicosane as main components, 9.60 and 4.61% respectively with lesser quantities of tetradecane, 1-bromo (0.44%), 2,4- pentadien-1-ol and 3-ethyl (0.29 %), and cycloheptasiloxane tetradecamethyl (0.26%). Other work from the southwestern part of Nigeria, the chemical composition of *Mitracarpus Scaber* leaf essential oil was investigated by gas chromatography – mass spectrometry (GC-MS). Twenty (20) compounds were identified in the essential oil accounting for 95.1% of the composition. The oil was dominated by the long-chain aldehyde pentadecanal (38.5%) and the polyunsaturated fatty acid ester methyl (7Z,10Z,13Z) hexadecatrienoate (11.8%), with lesser quantities of 2,3-dimethylnaphthoquinone (6.0%), and α-pinene (5.4%). The composition of *Mitracarpus Scaber* leaf oil in this work is notably different from a previous study [8], which showed to oil to be composed largely of saturated fatty acids (64.0%) and no monoterpene or sesquiterpene hydrocarbons.

Table 2: GC-MS analytical report of essential oil from *Mitracarpus Scaber* (Goga masu)

Peak	Compounds	Molecular Formula	Molecular Weight	Retention Time (Min)	Area (%)
1	2,4-Pentadien-1-ol, 3-ethyl-, (2Z)	C7H12O	112	9.833	0.29
2	Cycloheptasiloxane, tetradecamethyl	C14H42O7Si7	518	10.251	0.26
3	Tetradecane, 1-bromo-	C14H29Br	276	10.892	0.44
4	Methoxyacetic acid, 2-tridecyl ester	C16H32O3	272	11.601	0.50
5	Methoxyacetic acid, tetradecyl ester	C17H34O3	286	11.721	0.19
6	Hexadecane	C16H34	226	12.099	1.88
7	Hexadecane, 1,1'-oxybis-	C32H66O	466	15.223	0.98
8	Nonadecane	C19H40	264	15.401	3.39
9	3-(6,6-Dimethyl-5-oxohept-2-enyl)-cyclohexanone	C15H24O2	236	15.710	3.14
10	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (E)-(.+/-.)-	C15H28O	224	16.179	2.51
11	Eicosane	C20H42	282	16.396	3.60
12	Oxirane, tetradecyl	C16H32O	240	16.797	1.83
13	7Heneicosane	C21H44	296	17.363	4.61
14	Tetrapentacontane, 1,54-dibromo	C54H108Br2	914	17.644	4.54
15	Docosane	C22H46	310	18.273	2.64
16	Octadecane, 1-(ethenyloxy)-	C20H40O	296	18.588	1.61
17	Tricosane	C23H48	324	19.154	2.58
18	Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-	C13H21NO	207	19.778	2.65
19	Octadecane, 1-chloro-	C18H37Cl	288	20.001	2.97
20	Azetidine, 1-benzyl-3,3-dimethyl-2-phenyl-	C18H21N	250	20.459	9.60
21	Methoxyacetic acid, heptadecyl ester	C20H40O3	328	20.802	2.81
22	Oleic Acid	C18H34O2	282	21.123	3.05
23	2-Methylacetoxy-2-fluoro-1-phenylp	C11H11FO2	194	21.403	1.31
24	Hexadecenoic acid, Z-11-	C16H30O2	254	21.586	2.19
25	Pyrrole-2-carboxylic acid, 4-(1-ch lorodec-1-enyl)-3,5-dimethyl-,ethyl ester	C19H30ClNO2	339	22.153	3.42
26	6-Octadecenoic acid, (Z)-	C18H34O2	282	22.347	2.77
27	cis-Vaccenic acid	C19H36O2	296	22.713	3.45
28	Cyclododecanone, 2-(6-chloro-1-oxohexyl)-	C18H31ClO2	314	23.120	3.18

3.4 Antioxidant Activities of Crude Extract and Essential oil from *Mitracarpus scaber*

Natural antioxidants, presented as extract and essential oils, are characterized by a wide mechanism of action. Therefore, a single method of antioxidant activity is unable of comprehending the antioxidant profile, thus different assays of antioxidant activity should be used [5, 15]. Accordingly, in the present study the methanolic extract and essential oil from *Mitracarpus scaber* plant were examined for their free radical scavenging capacity towards the H_2O_2, Ferric Reducing Antioxidant Power and DPPH methods, which present different mechanisms of the determination of antioxidant capacity [1]. The percentage Scavenging capacity for plant extract, the essential oil and that of the standard (Ascorbic acid) were measured at varying concentrations (10, 20, 30, 40 and 50 µL). From the result all the samples have exhibited antioxidant activity as shown in table below.

A concentration-dependent scavenging activity was found for the studied samples which means that the average scavenging ratio for H_2O_2, FRAP and DPPH increases with the increasing concentrations of both extract, essential oil and the ascorbic acid (control).

In DPPH assay, the antioxidant activities of the extract ranged from 58.59 to 66.97%, essential oil ranged between 61.50 to 72.88% and the control ranged between 69.31 to 82.01%. The essential oil and the plant extracts were able to reduce the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow diphenylpicrylhydrazine with varying degrees of scavenging capacities. Great bleaching action (from purple to yellow) reflected a higher antioxidant activity (figure 1 to 3) [1]. The inhibition percent in the following order: Ascorbic acid (control) > Essential oil > Plants extracts.

All the samples scavenged H_2O_2 probably by donating electrons to the hydrogen peroxidase, thereby converting it into water. The H_2O_2 and FRAP scavenging activity of prepared extracts were found in the following order of essential oil > methanolic extract significantly different from the standard ascorbic acid. The maximum antioxidant activities by H_2O_2 and FRAP assay were observed at 50µL while the lowest were observed at concentration 10µL. It was noticed that the highest scavenging capacity the lowest

scavenging capacity for each of the samples were observed in their corresponding lowest concentrations. The % inhibition produced by ascorbic acid at concentration of 50µL was greater than the scavenging activities of each extract at a concentration of 50µL.

Table 3: Results of antioxidant activities of the methanolic extract of

Concentrations	H_2O_2	FRAP	DPPH
10 µg/ml	6.96	8.75	58.59
20 µg/ml	11.69	16.25	62.16
30 µg/ml	17.54	17.50	63.75
40 µg/ml	23.67	20.0	64.94
50 µg/ml	30.08	25.41	66.79

Table 4: Results of antioxidant activities of the Essential oil.

Concentrations	H_2O_2	FRAP	DPPH
10 µg/ml	56.54	8.33	61.50
20 µg/ml	63.50	13.33	63.62
30 µg/ml	65.73	22.91	65.21
40 µg/ml	67.96	27.91	66.40
50 µg/ml	73.81	35.0	72.88

Table 5: Results of antioxidant activities of Ascorbic acid (Control)

Concentrations	H_2O_2	FRAP	DPPH
10 µg/ml	62.39	11.25	69.31
20 µg/ml	64.62	16.67	72.08
30 µg/ml	67.68	31.67	75.66
40 µg/ml	71.58	37.92	78.04
50 µg/ml	76.60	52.08	82.01

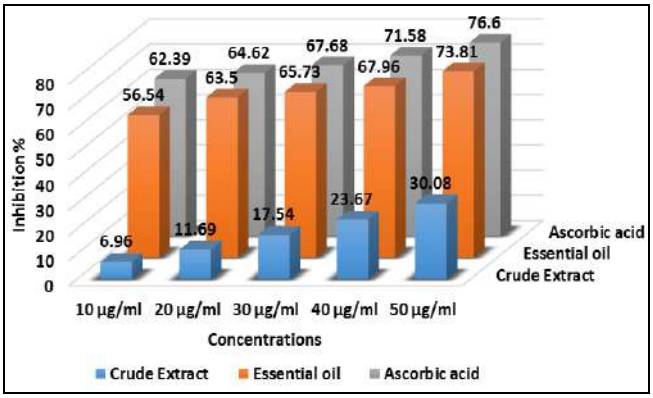

Fig 1: The *in vitro* antioxidant effect of the extracts, essential oil and Ascorbic acid using H_2O_2 assay

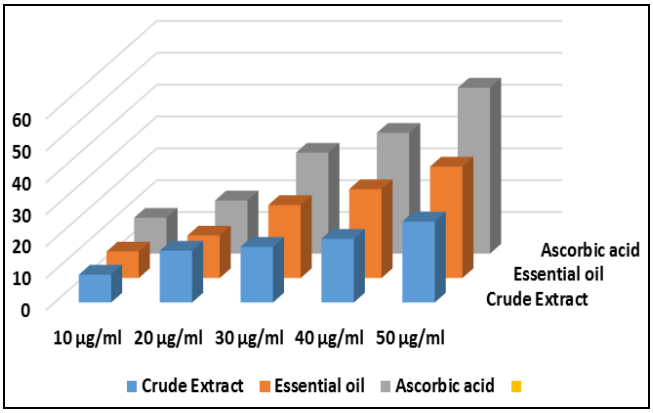

Fig 2. The *in vitro* antioxidant effect of the extracts, essential oil and Ascorbic acid using frap assay

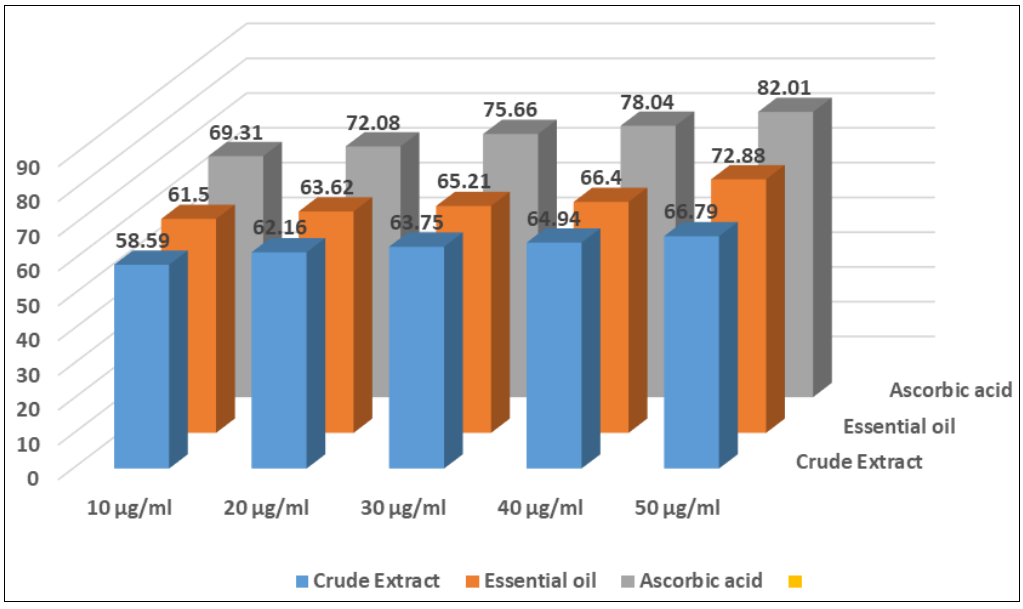

Fig 3: The *in vitro* antioxidant effect of the extracts, essential oil and Ascorbic acid using DPPH assay

Conclusion

The selected ethnomedicinal plants exhibited moderate to potent cytotoxicity against Artemia salina. Biopharmaceutical industries are in need of eco-friendly alternative drug molecules to treat diseases associated with microbial pathogens and body metabolism. Thus, methanolic extracts of *Ficus polita* and *Ficus thonningii Blume* plants might be a prospective source of alternative antimicrobial and antioxidant agents and may play an important role in the discovery of new drugs for the treatment of a wide range of pathogenic microorganisms in the near future. From the results obtained in this work, it can be concluded that all the extract and essential oil from *Mitracarpus scaber* plant contained therapeutic properties. The phytochemical results show that plant has naturally occurring phytochemical which have various application. The GC-MS analysis of the essential oils obtained from essential oil contained identifiable number of components which exceeded the number of components obtained in the same species grown in other countries or places as reported

by some authors, this could be due to variation in soil temperature and modification of the distiller which enable most of the volatile component of the plant to be condensed. The result of antioxidant activities of the essential oil and crude extract using DPPH, H_2O_2 and FRAP exhibited a great radical scavenging when compared to the antioxidant of ascorbic acid available in the market.

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