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Fatty Acid, Phospholipid and Sterol Compositions of Breadfruit (*Artocarpus altilis*) and Wonderful Kola (*Buchholzia aoriacea*) Seeds

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Abstract: A comprehensive study on fatty acid, phospholipid and phytosterol compositions of breadfruit (*Artocarpus altilis*) and wonderful kola (*Buchholzia coriacea*) seeds flour were determined using standard analytical techniques. The most concentrated fatty acid (%) was oleic acid in *Artocarpus altilis* seed (56.775) while linoleic acid (42.644) was the most concentrated acid in *Buchholzia coriacea* seeds. The increasing order of the concentrated fatty acids in *Artocarpus altilis* seeds were: stearic acid (4.723) < palmitic acid (11.412) < linoleic acid (25.710) < oleic acid (56.775) < while that of *Buchholzia coriacea* seeds were: linolenic acid (2.197) < stearic acid (6.734) < palmitic acid (11.241) < oleic acid (35.719) < linoleic acid (42.644), respectively. Arachidinic, linolenic, erucic, palmitoleic, behemic, lignoceric, arachidonic, margaric, myristic, lauric, capric, caprilic and caproic acids were present in small quantities with none of them recording up to 1.0% in both the two plant seeds. The results also showed high concentration of monounsaturated fatty acids (MUFA) (57.071%) in *Artocarpus altilis* and 36.739% in *Buchholzia coriacea*, and values of polyunsaturated fatty acids (PUFA) were 0.125 and 2.212% for the two plant seeds, respectively. The respective phospholipids composition of phosphatidylserine (204.75 mg/100g) and phosphatidylinositol (29.35 mg/100g) showed a highest concentration in *Artocarpus altilis* and *Buchholzia coriacea* while diphosphatidylglycerol was the least phospholipid with concentrations of 0.11 and 0.01 mg/100 g for both samples. The concentrations of phytosterols were of low values except in sitosterol with values of 90.81 and 31.24 mg/100 g in *Artocarpus altilis* and *Buchholzia coriacea* respectively. This study provides an informative oil profile that will serve as a basis for further chemical investigations and nutritional evaluation of the *Artocarpus altilis* and *Buchholzia coriacea* seed oils.

Keywords: Breadfruit, wonderful kola, fatty acids, phospholipids, phytosterols

Introduction

Plants serves as a primary source of food, medicines, fibres, shelters and other items used in everyday life by humans with roots, stems, leaves, flowers, fruit and seeds providing food for humans [1]. A large number of plant species are cultivated worldwide as ornamentals, living fences and firebreaks. They are also cultivated as soil binders, green manures, fodder for livestock, forage for honey bees, food for humans in agro forestry and reforestation (for nitrogen fixation), as pulp for paper production, fuel woods, timber, and as sources of chemicals and oils [2]. They serve as an indispensable constituent of human diet supplying the body with mineral salts, vitamins and certain hormone precursors, in addition to protein and energy [3]. Nutritive and calorific values of seeds make them necessary in diets [4, 5]. They represent a major direct source of food for man and livestock, and make a critical contribution to increased food security of subsistence farmers. Among these plant seeds are the seeds of breadfruit (*Artocarpus altilis*) and wonderful kola (*Buccholzia coriacea*).

Breadfruit (*Artocarpus altilis*) is an important food in the Pacific [6]. It is widely distributed in the tropics although native to Malaysia, Papua New Guinea and Philippines. Breadfruit trees grow easily in a wide range of ecological conditions with minimal input of labour or materials and require little attention or care [7]. It is high yielding with an average sized tree producing 400 – 600 fruits per year; whereas Morton reported yields between 16 and 32 ton/ha/year [8]. A single tree produces between 150 kg and 200 kg of fruits per season. However, the current usage, particularly, in developing countries, is limited by the poor fresh fruit storage properties. A few days after harvesting (3 days), the deterioration of the fruit settles. Because of their high water content they are

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easily susceptible to microbial attack as well as their bulky nature makes their transportation difficulty [9].

On the other hand, wonderful kola (*Buchholzia coriacea*) which is also known as 'musk tree' is a member of the family Capparaceae. It is an under storey forest tree with large, glossy, leathery leaves and conspicuous creamy white flowers. The species extends from Cote d'Ivoire to Gabon in Africa. The seeds of *B. coriacea* are edible and have medicinal value. Some researchers [10] assert that the seeds are used traditionally for treating diabetes, hypertension, rheumatism, cold, cough and catarrh. Some authors also contend that the stem and bark of the tree exhibited a high concentration dependent antibacterial and antifungal activity when subjected to methanol extract [11, 12]. There are about 2 – 3 seeds in a fruit. They are blackish with a spicy taste. The leaves are large and ellipsoid between 15 – 25 cm long and 5 – 7.5 cm broad [12]. The seed also acts as blood cleanser, facilitates learning ability and strengthens the nervous system. In Africa, the seed of *B. coriacea* is specially used against migraine and headache [13]. According to Sofowora, *Buchholzia coriacea* is known as 'uworol', 'owil' and 'uke' among Yoruba, Edo and Igbo ethnic groups of Nigeria [14].

This study is intended to give empirical information on the fatty acid, phospholipid and sterol compositions of breadfruit (*Artocarpus altilis*) and wonderful kola (*Buccholzia coriacea*). Such data will give information on the nutritive value of the plant seeds and will also be useful in evaluating the oils for other potential uses in food and industrial applications.

Materials and Methods

Samples collection and treatment

The fresh fruits of wonderful kola (*Buccholzia coriacea*) and breadfruit (*Artocarpus altilis*) were purchased from Ogoja market in Ogoja local government area of Cross River State, Nigeria and transported to the laboratory for treatment and analyzes. Four seeds each were removed from the fruits of *Buccholzia coriacea* and *Artocarpus altilis*, washed, peeled and dried in an oven at 45^{o}C for 72 h. The dried seeds were ground into powder separately using a food blender, sieved through a 250 µm and then stored in a separate airtight container for further analysis.

Extraction of oils

Each sample of wonderful kola and breadfruit was oven dried and extracted in Soxhlet apparatus with redistilled n–hexane of Analar grade (British Drug Houses, London) for the recovery of undiluted oil. The crude oil extract was made to be free of water by filtering through the anhydrous sodium sulphate salt. The hexane was removed from the oil/hexane mixture by using a rotary evaporator.

Fatty acid analysis

The oil extracted from each sample was converted to the methyl ester using the method described by Akintayo and Bayer [15]. About 2 mg crude oil sample was transferred into a 5 – 10 mL glass vial and 1 mL of diazomethane ether solution added. The mixture was shaken thoroughly and allowed to stand for 1 min. Then 16 µL of 3.33 M CH_3CONa/CH_3OH solution was added; mixture shaken and allowed to stand for 10 min after which 10 µL acetic acid was added. The fatty acid methyl esters were analyzed using a HP 6890 gas chromatograph powered with HP Chemistation Rev. a 09.01 (1206) software fitted with a flame ionization detector and a computing integrator. Nitrogen was used as the carrier gas. The column initial temperature was 250^{o}C rising at 5^{o}C/min to a final temperature of 310^{o}C while the injection port and the detector were maintained at 310 ^{o}C and 350 ^{o}C, respectively. A polar (HP INNO Wax) capillary column (30 m x 0.53 mm x 0.25 µm) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl esters obtained from Sigma Chemical Co. (St. Louis MO, USA).

Phospholipids analysis

The analysis of the extracted oil phospholipids content was determined a follows: 0.01 g of the extracted fats was added to the test tube. To ensure complete dryness of the oil for phospholipids analysis, the solvent was completely removed by passing the stream of the nitrogen gas on the oil. 0.04 mL of chloroform was added to the content of the tube and it was followed by the addition of 0.10 mL of chromogenic solution. The content of the tube was heated at a temperature of 100^{o}C in a water bath for about 1 min. The content was allowed to cool, 5 mL of the hexane was added and the tube with its content shook gently several times. The solvent and the aqueous layers were recovered and allowed to be separated. The hexane layer was recovered and allowed to be concentrated to 1.0 mL for gas chromatography using flame photometric detector. The conditions for phospholipid analysis include H.P 5890 powered with HP ChemStation REV. A 09.01 (1206) and split injection ratio of 20: 1; nitrogen as carrier gas; inlet temperature, 250^{o}C; column type, HP5; column dimension: 30 m x 0.25 mm x 0.25 µm; oven program: Initial temperature at 50^{o}C; first ramping at 10^{o}C/min for 20 min, maintained for 4

min while second ramping at 15°C/min for 4 min, maintained for 5 min. Detector: PFPD Detector temperature: 300^{0}C; hydrogen pressure, 20 psi; compressor air: 35 psi.

Phytosterol Analysis

The aliquots of the extracted fat were added to the screw – capped test tubes. The samples were saponified at 90 °C for 30 min, using 3 ml of 10 % KOH in ethanol, to which 0.20 mL of benzene had been added to ensure miscibility. The deionized water (3 mL) was added and 2 mL of hexane was added in extracting the non – saponifiable materials. Three extrations, each with 2 mL of hexane were, carried out for 1 h, 30 min and 30 min, respectively. The hexane was concentrated to 1 mL in the vial for gas chromatography analysis and 1 µL was injected into the injection pot of GC. The GC conditions of analyses were similar to the GC conditions for methyl esters analyses.

As for the purpose of ensuring the accuracy of the results obtained and quantification the following were done: Standard chromatograms were prepared for fatty acid methyl esters, phospholipids and phytosterols which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient determined for fatty acids, phospholipids and sterols. Correlation is a statistical index that shows the quality assurance of the calibration curve performed and it was prepared with the Howlett Packard Chemistry (HPCHEM) software (GMI, Inc 6511 Bunker Lake Blud Ramsey, Minnesota, 55303, USA).

Results and Discussion

The percentage fatty acid composition of *Artocarpus altilis* and *Buchholzia coriacea* seeds are shown in Table 1. The results showed that oleic acid (C18:1) and linoleic acid (C18:2) formed the first and second most abundant fatty acids in *Artocarpus altilis* whereas reverse is the case in *Buchholzia coriacea.* The result of oleic and linoleic acids in the present study are comparably higher than oleic (12.4 and 14.29 mg/100g) and linoleic (14.8% and 33.27 mg/100g) in *Artocarpus altilis* and *Buchholzia coriacea,* respectively as reported by some workers [16, 17]. This is in agreement with the report of Grosso *et al.* [18], that linoleic and oleic acids are major fatty acids in many plant seeds such as peanut, soybean, chide pea, garden pea, broad bean and lentil. Oleic has been regarded as monounsaturated fatty acid and has been shown to decrease HDL-cholesterol concentrations which affect positively cardiovascular disease risk [19]. This report on *Artocarous altilis* gives result of oleic acid slightly higher than the values reported for African locust and mesquite bean (32.24% and 30.96%, respectively) [20]. The linoleic values in both samples are comparable with values obtained for linoleic in *Luffa cylindrical* and *Brachystegia eurycoma* as reported by some Researchers [21, 22]. It is increasingly recognized that an insufficient intake of omega-6 acid such as linoleic causes growth retardation in children, heart attack risk and skin ailments [23]. Palmitic acid (C16:0) is third in concentration (Table 1), with values of 11.412 and 11.241% for *Artocarpus altilis* and *Buchholzia coriace,* respectively. The values obtained in this study are lower compared to 21.4% and 30.28 – 35.77% obtained for *Artocarpus altilis* and *Buchholzia coriace*, respectively [16, 17]. It has been reported that many lipids contain substantial amounts of saturated fatty acids especially palmitic acid. Stearic acid (C18:0) (4.723 and 6.734%) takes the fourth position in both samples of *Artocarpus altilis* and *Buchholzia coriacea.* It is slightly higher when compared with 2.0% of the stearic acid value for *Artocarpus altilis* [16]. A higher proportion of either linoleic or oleic acid is associated with legumes containing insignificant lipids [24]. Lignoceric, behenic, arachidonic, erucic, arachidic, margaric, myristic, lauric, capric, caprylic and butyric acids contained some percentage of fatty acid less than 1%.

Table 1: Fatty acid composition of *Artocarpus altilis* and *Buchholzia coriacea* seed oils

Name	***Artocarpus altilis***	***Buchholzia coriacea***
Butyric Acid (C4: 0)	<0.001	<0.001
Caproic Acid (C6: 0)	0.009	<0.001
Caprylic Acid (C8: 0)	0.007	<0.001
Capric Acid (C10: 0)	0.050	<0.001
Lauric Acid (C12: 0)	0.031	<0.001
Myristic Acid (C14: 0)	0.123	<0.001
Palmitic Acid (C16: 0)	11.412	11.241
Margaric Acid (C17: 0)	0.015	0.061
Stearic Acid (C18: 0)	4.723	6.734
Arachidic Acid (C20: 0)	0.185	0.038

Behenic Acid (C22: 0)	0.140	0.251
Lignoceric Acid (C24: 0)	0.393	0.080
Palmitoleic Acid (C16:1)	0.290	0.756
Erucic Acid (C22:1)	0.006	0.264
Oleic Acid (C18:1)	56.775	35.719
Linoleic Acid (C18:2)	25.710	42.644
Linolenic Acid (C18:3)	0.125	2.197
Arachidonic Acid (C20:4)	0.004	0.015

Table 2: Fatty acid distribution of *Artocarpus altilis* and *Buchholzia coriacea* seed oils according to degree of saturation and unstauration of the component

Parameter	*Artocarpus altilis*	*Buchholzia coriacea*
TSFA	17.089	18.411
TSFA (%)	17.089	18.411
MUFA	57.071	36.739
DUFA	25.710	42.644
PUFA	0.125	2.212
TUFA	82.910	81.595
TUFA (%)	82.910	81.595
TEFA	25.835	44.841
TNEFA	74.165	55.159
O/L	2.208	0.838

TSFA = Total saturated fatty acid, TUFA = Total unsaturated fatty acid, TEFA = Total Essential fatty acid, DUFA = Diunsaturated fatty acid, MUFA =Monounsaturated fatty acid, O/L =Oleic/Linoleic ratio

The fatty acid distribution according to saturation and unsaturation was shown in Table 2. The total saturated fatty acids (TSFA) were 17.089% for *Artocarpus altilis* and *Buchholzia coriacea* (18.411%) These values are lower than TSFA values of 20.50% and 24.80% reported for boiled and raw tigernuts samples [25], 54.51% reported for dehulled African yam bean [26], 34.68% reported for bambara groundnut [27], 40.20% and 43.00% reported for African locust bean and mesquite bean, respectively [20]. The reported values of 12.3% for groundnut [28], 15.2% for soybean [29], 9.0-12.9% for pinto bean [30], and 17.06% for *B. eurycoma* [22] are lower than the values reported in this work. However, the values revealed for the total unsaturated fatty acid (TUFA) were 82.910% and 81.595% in *Artocarpus altilis* and *Buchholzia coriacea* as shown in Table 2 are higher than that of tigernut sample with 75.20% (in raw) and 79.50% (in boiled) [25], *Parkia biglobosa* with a reported value of 33.69% [31], *Adenanthera pavoninawith* a reported value of 66.67% [32], bambara groundnut with a reported value of 65.32% [27] and mesquite bean with a reported value of 56.90% [20]. Total unsaturated fatty acid (TUFA) in this study is of good concern because report has shown that fats and oils with high unsaturation are particularly susceptible to oxidation and intakes of food containing oxidized lipid increase the concentration of secondary proxidation products in liver [33]. High amount of the total unsaturated fatty acid (TUFA) makes *Artocarpus altilis* and *Buchholzia coriacea* as a special seeds for nutritional applications. These findings imply that *Artocarpus altilis* and *Buchholzia coriacea* seed oils are as good as soybean and cowpea seed oils in the supply of essential fatty acids. Linoleic and alpha-linolenic acids called omega-6-fatty acids and omega-3-fatty acids, respectively are the most important essential fatty acids required for growth, physiological functions and body maintenance [24]. These two fatty acids work together in competitive balance to regulate blood clotting, immune response and inflammatory processes. Deficiency of linoleic acid leads to dry hair, hair loose [34] and poor wound healing [35]. It also leads to poor growth, fatty liver, skin lesion and reproductive failure [36]. It has been reported that linoleic acids plays a role in lowering the risk of cardiovascular disease [37]. It has also been found that the intake of linoleic aicd in the diet protects against fatal schemic heart disease [38]. Wonderful cola (*Buchholzia coriacea*) had the highest TEFA (44.841) content. The oleic/linoleic (O/L) acid ratio has been associated with high stability and potentiality of the oil for deep frying fat [39]. The O/L level is 2.208 in *Artocarpus altilis* and 0.838 in *Buchholzia coriacea* seeds. These values are lower than that of *Anarcadium occidentale (*12.28%) [40], but compared with that of tigernut 0.69% (in boiled) and 2.11% in (raw) [25] and 1.48% for peanut oils [40] hence *Artocarpus altilis* may be stable compared with peanut oil and may be useful as frying oil. Monounsaturated fatty acid (MUFA) values were

57.071% and 36.739% in *Artocarpus altilis* and *Buchholzia coriacea*, respectively; polyunsaturated fatty acid (PUFA) value in *Artocarpus altilis* was 0.125% and 2.212% in *Buchholzia coriacea* while diunsaturated fatty acid (DUFA) values for both the samples *Artocarpus altilis* and *Buchholzia coriacea* were 25.710% and 42.644%. Linoleic acid constituted the DUFA while total nonessential fatty acid (TNEFA) gave 74.165 and 55.159% for *Artocarpus altilis* and *Buchholzia coriacea* seeds, respectively.

Table 3 shows the phospholipids content of *Artocarpus altilis* and *Buchholzia coriacea* seeds. From the result phosphatidylserine (204.75 mg/100 g) and phosphatidylinositol (29.35 mg/100 g) showed greater concentrations in *Artocarpus altilis* and *Buchholzia coriacea*, respectively. Phosphatidycholine and phosphatidyethanolmine came second with values of 195.03 mg/100g and 23.45 mg/100 g for both *Artocarpus altilis* and *Buchholzia coriacea*. Phosphatidylinsitol in the case of *Artocarpus altilis* followed Phosphatidycholine with the value (59.87 mg/100 g) and (19.41 mg/100 g) value of phosphatidyserine followed phosphatidyethanolmine for *Buchholzia coriacea* shown in Table 3. The fourth and fifth most concentrated phospholipids in *Artocarpus altilis* were phosphatidic acid and phosphatidyethanolmine with concentrations of 27.51 mg/100 g and 21.12 mg/100 g. Phosphatidycholine, lysophosphatidycholine, phosphatidic acid, and diphosphatidylglycerol were the minor phospholipids with concentrations of 8.58 mg/100g; 8.51 mg/100 g; 9.24 mg/100 g; 0.005 mg/100 g for *Buchholzia coriacea* while diphosphatidylglycerol and lysophosphatidycholine were the minor phospholipids with concentrations of 0.11 mg/100 g and 8.72 mg/100 g for *Artocarpus altilis* seed flour. Contrary to the report of Wirtz [41], phosphatidythanolamine usually the most abundant phospholipid in animals and plants, often amounting to almost 50% of the total and as such they are building block of membrane bilayer.

Table 3 Phospholipids composition of *Artocarpus altilis* and *Buchholzia coriacea* seed oils

Name	*Artocarpus altilis*	*Buchholzia coriacea*
Lysophosphatidylcholine	8.72	8.51
Phosphatidylethanolamine	21.12	23.45
Phosphatidylcholine	195.03	8.58
Phosphatidylglycerol	19.12	0.36
Phosphatidylserine	204.75	19.41
Phosphatidylinositol	59.87	29.35
Diphosphatidylglycerol	0.11	0.01
Phosphatidic acid	27.51	9.24

The phosphatidycholine value of *Artocarpus altilis* is high. This may be as a result of the shelf life of the seed, because researchers had found that phosphatidycholine concentration is high at infancy but slowly depletes throughout the age of life, and may drop to as low as 10% of the cellular membrane in the elderly plants and animals [42]. As a result of this, researchers have recommended daily supplementation of phosphatidycholine as a way of improving brain functioning memory capacity [43]. The US Food and Drug Administration (USFDA) has stated that consumption of phosphodyserine may reduce the rate of dementia and cognitive dysfunction in the elderly people, in young people it reduces mental stress and increases mental accuracy and stress resistance [44]. Phosphodyserine supplementation promotes a desirable hormonal balance for athletes and might reduce the physiological detorations that accompanies over training and/or overstretching [45]. Phosphatidic mediates cellular functions through different modes of action, such as membrane tethering, modulation of enzymatic activities and structural effects on cell membranes. The regulatory processes in which phosphatidic plays a role include; signaling pathways in cell growth, proliferation, reproduction and responses to hormones in biotic and abiotic stress [44]. Therefore, consumption of these plant seeds particularly *Artocarpus altilis* may participate well in these functions. From the result *Artocarpus altilis* has more concentrated values of phospholipids than *Buchholzia coriacea* seed, consequently *Artocarpus altilis* can be regarded as a better source of phospholipids as compared to *Buchholzia coriacea*.

The composition of phytosterols in *Artocarpus altilis* and *Buchholzia coriacea* seeds were presented in Table 4. The results are in agreement with that recorded for many oils where β-sitosterol (90.81% & 31.24%) constitutes the major phytosterol follow-up by stigmasterol (5.43% & 5.00%) [46, 47] report on by [25]. In the same way, the total phytosterols (97.17 mg/100g) for *Artocarpus altilis* is similar to those of other edible oils [48, 49]. The values for

savenasterol, campesterol, ergosterol, choslestanol and cholesterol for the samples ranged between 0.93 - 0.49%; 4.24 – 10.19%; 4.56e-4 - 1.85e-3%; 4.57e-4 – 1.99e-5; and 3.52 – 2.40e-5 respectively. This result showed that *Artocarpus altilis* can be regarded as a better source of phytosterols when compared to *Buchholzia coriacea.* Phytosterols are natural components of plant origin forming cell membrane and occur in small quantities in many fruits, vegetables, nuts, seeds, cereals, legumes, vegetable oils and other plants. They are abundantly present in the fat soluble fractions of all the plants and food containing plant based raw materials including principally oils, cereals, pulse and dried fruits [50]. Phytosterols may exist as free sterols (FS's), esterified with fatty acids (SE's) or phenolic acids (SPEHE's) or glycosides (SG's) and acylated glycosides [51]. Systematic reviews studying the efficacy of phytosterols have shown that phytosterols enriched foods can significantly lower LDL cholesterol [52]. Plant phytosterols have also been described as anti-inflammatory and anti-cancer compounds [53, 54]. Daily intake of phytosterols helps to prevent heart disease by lowering HDL cholesterol levels by as much as 14% [55]. A summary of approximately 52 studies revealed that an average of 13±1 g of phytosterols intake daily for 3 – 5 weeks showed a 20% decrease in blood cholesterol level [56]. Through competition of phytosterols with cholesterol absorption and uptake in the small intestine the supply of cholesterol has greatly reduced. This process of cholesterol reduction as a result of uptake of phytosterols in turn reduces the risk of heart disease (CHD) since high blood total cholesterol and low-density lipoprotein (LDL) cholesterol levels are the main risk factors for CHD [25]. Phytosterols have been found useful in treating other conditions, including rheumatoid arthritis, but their widest application is in protecting the heart [57]. However reports also suggest that excessive intake of dietary phytosterols and stanols in plasma and tissues may contribute to the increased blood pressure [57].

Table 4. Phytosterols Composition of *Artocarpus altilis* and *Buchholzia coriacea* seed oils

Name	*Artocarpus altilis*	*Buchholzia coriacea*
Cholesterol	3.52e-4	2.40e-5
Cholestanol	4.57e-4	1.99e-5
Ergosterol	4.59e-4	1.85e-3
Campesterol	4.24	10.19
Stig-masterol	5.43	5.00
Savenasterol	0.93	0.49
Sitosterol	90.81	31.24

Conclusion

The present work has focused on the lipid composition of *Artocarpus altilis* and *Buchholzia coriacea* seeds. The work revealed that the oils contained high proportion of unsaturated fatty acids and significant contents in phospholipids and phytosterols. In summary, this study indicates that *Artocarpus altilis* seeds have high oil content compared to *Buchholzia coriacea* seeds and therefore could be exploited as a natural source of edible oil. *Artocarpus altilis* oil was a rich source of unsaturated fatty acids with potential beneficial therapeutic activities. This study provides an informative lipid profile that will serve as a basis for further chemical investigations and nutritional evaluation of the *Artocarpus altilis* and *Buchholzia coriacea* seed oils.

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