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Extraction, Characterization and Evaluation of the Antioxidant Activity of Ximenia Americana in Sudan

Wafa Mohammed Hussein Abuelgasim[1], Ahmed Mohamed Farah Shadoul[2], Feisal Monowar Ahmed Esmeil[3], Fawzia Ahmed Salih Fadl Allah[4], Ayman Anwar Mohammed osman[5], Muslih Haroun Elhussein Gamea[6*], Ahmed Mohamed Zain[7]

[1]Faculty of medical laboratory Sciences, Assistant professor of medical microbiology, National Ribat University
[2]Secretary General Sudan Medical Specialization Board, Sudan
3 Associate professor, Consultant of Orthopedic and Traumatology, Sudan
[4]Dongola university, assistant Professor of biology, Sudan
[5]Forensic Science Institute, National Ribat University. Sudan
[6]Sudan International University, Register of the National Council for Medical and Health Professions, Sudan
[7]Dongola university, Professor of Microbiology, Sudan

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*Corresponding Author: Muslih Haroun Elhussein Gamea Email: muslihlab0900@gmail.com

Abstract: **Background**: Ximenia americana (wild plum), a shrub belonging to the Olacaceae family and native to tropical Africa, has long been used in traditional medicine for treating skin conditions, infectious diseases, and inflammatory disorders. The seeds of this plant contain a substantial amount of oil (approximately 27.71%) that is widely used in African communities for cosmetic and medicinal purposes. However, comprehensive scientific characterization of the seed oil and systematic evaluation of its antioxidant potential remain limited especially in Sudan. **Methods**: This study was designed to extract oil from X. americana seeds, characterize its physicochemical properties, and evaluate its antioxidant activity in Sudan. The oil was extracted using standard solvent extraction methods. The physicochemical properties—including color, odor, taste, boiling point, density, viscosity, refractive index, acid value, ester value, iodine value, peroxide value, and saponification value—were determined following standard protocols. The antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Chemical constituents of the ethanol extract were identified by gas chromatography coupled to mass spectrometry (GC-MS) following saponification and methylation of fatty acid components. Phytochemical screening was performed to detect secondary metabolites including phenols, flavonoids, tannins, saponins, terpenoids, and alkaloids. **Results**: The GC-MS analysis successfully identified ten fatty acids in the seed oil, including four previously reported acids (palmitic, oleic, ximeninic, and lignoceric) and six additional acids (ceric, nervonic, montanic, ximenic, ximenoic, and lumequeic). Notably, ximeninic acid and ximenoic acid are unique long-chain fatty acids characteristic of this species. The seeds exhibited high protein content (19.77%) and oil content (27.71%). The oil was yellow, odorless, tasteless, and freely soluble in non-polar solvents. Key physicochemical parameters were: boiling point 157°C, density 0.9376 g/ml, refractive index 1.4770, acid value 0.2805, ester value 9.82, iodine value 47.59, peroxide value 30, and saponification value 11.43. The phytochemical screening revealed the presence of phenols, flavonoids, tannins, and other bioactive compounds that contribute to the oil's antioxidant and medicinal properties. The DPPH assay confirmed significant free radical scavenging activity, supporting the traditional use of the oil for therapeutic and cosmetic applications. **Conclusion**: The findings provide substantial scientific evidence that Ximenia americana seed oil is a rich source of bioactive fatty acids and phenolic compounds with notable antioxidant efficacy. The high oil and protein content, combined with the presence of unique ximeninic and ximenoic acids, underscore the potential of this plant as a valuable resource for nutraceutical development, cosmetic formulations, and possibly biodiesel production. Further purification and structural elucidation of the active phenolic compounds are recommended for the development of novel phytopharmaceuticals and therapeutic agents.

Keywords: Ximenia americana, Sudan, seed oil, gas chromatography-mass spectrometry, antioxidant activity, DPPH assay, fatty acids, phytochemicals, ximeninic acid, traditional medicine, nutraceuticals.

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1.1 INTRODUCTION

Ximenia (*Ximenia americana* L.), known as wild plum, is a shrub, or small tree, belonging to the Olocaceae family. This shrub, native from tropical Africa, is nowadays spread across different continents and can be found in the Western Islands, Pacific Islands, New Zealand, and Central and South America. It is an evergreen tree providing flowers and fruits all year. It is drought resistant and grows in poor soils. The fruit has a large kernel that contains about 60% in oil [1]. Several studies have been published showing the medicinal potential of this plant, such as for the treatment of the skin [2], as antimicrobial [3], as gastroprotective action [4], and antioxidant activity [5].

Native populations use the wood as fuel, to produce long sticks appropriate to mix food while cooking and also to produce other domestic objects like spoons and handles for scythes because of its strength and attractive appearance. However, the most valuable part of this tree is the oil obtained from its seeds.

In southwest Africa, the oil is extensively used by local populations, mainly as a skin cosmetic: to smooth and hydrate, to give a more pleasant color, to increase skin elasticity, to prevent stretch marks in pregnant woman, and as a hair conditioner [6]. The oil is also used as a folk medicine. Massage with the oil is claimed to cure arthritis and abdominal pains and to prevent the onset of varicose veins. There is some controversy about the edibility of the oil extracted from seeds. For instance, in Niger some authors considered it edible [7]. It was also reported that the oil is used in some African countries to prepare food [8]. However, some authors [9] were disagreeing with this use because of its physicochemical characteristics, namely the high peroxide content and the low saponification and iodine values.

In African rural communities are dependent on cattle breeding for survival and to get some income to acquire industrial goods. In these societies, the possibility to get extra income collecting and selling forest products other than wood is very important. In this context, Ximenia americana oil is traded in the local markets since it is appreciated as a skin cosmetic. It is also commercialized in urban markets, improving the economic sustainability of the populations that produce it [6].

The extraction of the oil by African woman has recently been reported [6, 8] and comprises several steps: drying the fruits, toasting the seeds, gridding the toasted seeds, and drying the resulting mass at the sun. Then this mass is toasted again and, after that, is cooked with hot water until the oil separates. The mixture is cooled to room temperature and the oil is collected by woman. The oil is again warmed during an established time and, after cooling, is placed in a container to be sold.

Considering our study was aimed the identification of X. americana seed oil components by gas chromatography coupled to mass spectrometry (CG / MS), components of the oil were converted to esters of fatty acids by means of methylation reaction. Basically, two reactional pathways have been most frequently used to convert fatty acids to methyl esters: mild alkaline methanolysis and saponification followed by fatty acid methylation (saponification/methylation). In the end detects the antioxidant activity after characterization of Ximenia Americana seed oil.

1.2 Problem identification and Justification:

Since ancient times, medicinal plants have been traditionally used for treatment of different diseases. Nowadays plants are considered a valuable source of unique natural compounds used in development of antidiabetic, anticancer and antimicrobial drugs, therefore the present study was characterized and evaluated the antioxidant activity of Ximenia Americana activity and measured by using Gas chromatography–Mass spectrometry analysis in Sudan.

Objectives:

1.3.1 General objective:

1.3.2 Extraction, Characterization and Evaluation of the Antioxidant Activity of Ximenia Americana in Sudan.

1.3.3 Specific objectives:

1.3.3.1 To extracted of **Ximenia** Americana seed oil.
1.3.3.2 To estimated physiochemical properties of Ximenia Americana seed oil.
1.3.3.3 To detected antioxidant activity of Ximenia Americana by used a DPPH free radical activity.
1.3.3.4 To identified a chemical constituent from the ethanol extract of Ximenia americana seed oil and to study their radical scavenging.

2. LITERATURE REVIEW

Widad, *et al.* [10] were annotated the phytochemical constituents of a root extract from *Ximenia americana* var. *caffra* and highlighted its hepatoprotective and hypoglycemic properties. We here extended our study on the leaf extract and identified its phytoconstituents using HPLC-PDA-ESI-MS/MS. In addition, we explored its antioxidant, antibacterial, and antiaging activities *in vitro* and in an animal model, *Caenorhabditis elegans*. Results from HPLC-PDA-ESI-MS/MS confirmed that the leaves contain 23

secondary metabolites consisting of condensed tannins, flavonol glycosides, flavone glycosides, and flavonol diglycosides. The leaf extract demonstrated significant antioxidant activity *in vitro* with IC_{50} value of 5 μg/mL in the DPPH assay and 18.32 μg/mL in the FRAP assay. It also inhibited four enzymes (collagenase, elastase, hyaluronidase, and tyrosinase) crucially involved in skin remodeling and aging processes with comparable activities to reference drugs along with four pure secondary metabolites identified from the extract. In accordance with the *in vitro* result, *in vivo* tests using two transgenic strains of *C. elegans* demonstrated its ability to reverse oxidative stress. Evidence included an increased survival rate in nematodes treated with the prooxidant juglone to 68.9% compared to the 24.8% in untreated worms and a reduced accumulation of intracellular reactive oxygen species (ROS) in a dose-dependent manner to 77.8%. The leaf extract also reduced levels of the expression of HSP 16.2 in a dose-dependent manner to 86.4%. Nuclear localization of the transcription factor DAF-16 was up to 10 times higher in worms treated with the leaf extract than in the untreated worms. The extract also inhibited the biofilm formation of *Pseudomonas aeruginosa* (a pathogen in skin infections) and reduced the swimming and swarming mobilities in a dose-dependent fashion. In conclusion, leaves of *X. americana* are a promising candidate for preventing oxidative stress-induced conditions, including skin aging.

Reactive oxygen species (ROS) are an integral part of normal physiological processes, continuously formed as a consequence of aerobic metabolism in eukaryotic cells. ROS at low-to-moderate concentrations play an important role in cell physiology, such as regulation of cell growth, cellular signal transduction pathways, and defense against pathogens [11, 12]. In addition to their biological importance, overproduction of these extremely reactive and unstable oxygen species is considered to be the main contributor to various metabolic and cellular disturbances. Oxidative stress has been suggested to play a major role in the pathogenesis of many degenerative diseases in humans [13], i.e. inflammatory, cancer, diabetes, aging, cardiovascular diseases; tumor growth and Alzheimer's disease are contributed by increased cell oxidation [14-17]. In modern medicine, maintaining the balance between antioxidant defense system and ROS formation is believed to be a critical concept for healthy biological systems [18]. However, in recently published data there is use of synthetic antioxidants like hydroxyl toluene, butylated hydroxyanisole, tert-butylhydroquinone and propyl gallate are widely used in the food industry to prevent oxidative deterioration [19]. However, it has been established that synthetic antioxidants appear to have carcinogenic and tumor-promoting action [20]. Therefore, it is of great importance to find new sources of safe and inexpensive antioxidants of natural origin in order to use them in food and pharmaceutical formulations. Several studies have showed that increased dietary intake of natural phenolic antioxidants correlate with decreased coronary heart disease [21]. Natural products-based drugs have used against various diseases since time immemorial. Plant derived natural products hold great promise for the discovery and development of new drugs. Studies have been conducted to identify antioxidants from natural sources which are suitable alternatives to synthetic antioxidants [22, 23]. Polyphenolic compounds or phytochemicals present in plants are important components of the human diet; Phytochemicals can be used to regulate oxidation and stress-related chronic diseases such as diabetes and cardiovascular diseases [24]. The protective effects of phenolic compounds and flavonoids are directly related to their ability to scavenge free radicals [25] by undergoing oxidation, producing toxic compounds, which elicit inhibitory effects on pathogenic microorganisms [26]. In order to protect the human body from free radicals, a number of studies have been carried out on various plants, vegetables and fruits which are a rich source of antioxidants, such as vitamin A, vitamin C, polyphenolic compounds and flavonoids [27]. Inflammation is a very common symptom of many chronic diseases and it is normal protective response to tissue injury caused by chemical or microbial agents [28]. It is well known fact that denaturation of tissue proteins leads to inflammatory and arthritic diseases [29]. Inflammation plays an important role in various diseases, such as rheumatoid arthritis and asthma. During an inflammatory response, mediator such as cytokines, interleukin-1, tumour necrosis factor and interferons is released [30, 31]. Since ancient times, in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant derived formulations. An anti-inflammatory activity of several plant extracts and isolated compounds has already been scientifically demonstrated [32, 33].

Ximenia americana (X. americana) belonging to Olacaceae family was selected because it is used in the treatment for a wide variety of ailments by many rural communities in Asia commonly known as "wild olive". It is extensively used as herbal remedy in treatment of malaria, leproutic ulcer, skin infections [34], as antibacterial activity, in fever, tuberculosis, stiffness, tooth decay and wounds [35]. Many works have reported the use of roots in the treatment of leprosy, syphilis, dysentery, and wounds. The stem bark has been reported to have anti-trypanosomal activity and used in treating headaches and mumps [36]. X. americana species, where systematic study is still not satisfactory, specially, is relative to specific biological activity of their chemical constituents. However, this plant has not been studied for anti-inflammatory activity and there are very few available literatures on antioxidant activity of this plant were found.

2.1 Taxonomy of Ximenia americana

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae – green plants
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida
Superorder	Santalanae
Order	Santalales
Family	Ximeniaceae
Genus	Ximenia L.
Species	Ximenia americana L. – tallow wood, tallowwood

The genus Ximenia belongs to the Olacaceae and comprises about 8 species [37]: Ximenia roiigi, Ximenia aegyptiaca, Ximenia parviflora, Ximenia coriaceae, Ximenia aculeata, Ximenia caffra, Ximenia americana and Ximenia aegyptica. X. caffra stands out for being used in Tanzania for the treatment of irregular menstruation, rheumatism and cancer [38] and, in Limpopo Province, South Africa, for treatment diarrhea [39]. However, X. americana Linn. is the most common, being native to Australia and Asia where is commonly known as Yellow Plum or Sea Lemon. It is found mainly in tropical regions (Africa, India, New Zealand, Central America and south America), especially Africa and Brazil. The plant is characterized as a small tree spinose 3-4 feet tall, gray or reddish bark, with leaves small, simple, alternate, of bright green color and with a strong smell of almonds. The flowers are yellowish-white, curved and aromatic. Fruit is yellow-orange, aromatic, measuring 1.5 to 2.0 cm in diameter, surrounding a single seed and have a pleasant plum-like flavor [40]. In Asia, the young leaves are consumed as a vegetable, however, the leaves also contain cyanide and need to be thoroughly cooked, and should not be eaten in large amounts.

2.2 Biological activity:

2.2.1 Antimicrobial and antifungal activities:

To evaluate the scientific basis for the use of numerous plants species used to treat diseases of infectious origin, crude extracts of these plants were investigated. The antimicrobial activity of the extracts of the various parts of the investigated plants such as roots, leaves, seeds, stem barks and fruits, appears to be due to the presence of secondary metabolites such polyphenols, triterpenes, sterols, saponins, tannins, alkaloids, glycosides and polysaccharides [41-44]. X. americana is a plant used in traditional medicine for the treatment of malaria, leproutic ulcers and infectious diseases of mixed origin by natives in Ethiopia, Guinea, Sudan and in the Northern part of Nigeria [41, 42, 44-47]. The crude extracts of X. americana showed antimicrobial and antifungal activities. The crude aqueous, methanolic, ethanolic, butanolic and chloroform extracts from different parts (leaves, root, stem and stem bark) of the plant were subjected to phytochemical screening and from the test carried out, it was observed that the secondary metabolites contained were saponins, flavonoids, tannins, terpenoids, sterols, quinones, alkaloids, cyanogenetic glycosides, cardiac glycosides and carbohydrates in the form of sugars and soluble starch. The MeOH extract from leaves of X. americana inhibited or retarded growth of *Neisseria gonorrhea* organism at dilution as low as 250 µg/ml. This same extract showed antifungal effect against *Candida albicans* and *Cryptococus neoformans* in concentration of 4000 µg/ml. Chemical screening conducted on the extract showed the presence of several secondary metabolites as tannins, sterols, terpenoids, flavonoids and saponins [41]. The antimicrobial activities of ethanol extract of the leaves were evaluated against six common bacterial isolates (*Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Escherichia coli, Staphylococus aureus* and *Candida albicans*) and was active against all of them. The highest degree of activity was for P. aeruginosa (inhibition zone: 20 mm), followed by B. subtilis and C. albicans (inhibition zone: 10 mm). Activity of the organic extract of the plant was comparable to that of commercially available penicillin disc (2 µg) which was more active against P. aeruginosa but less effective against S. aureus. The results of phytochemical analysis indicated the presence of saponins, flavonoids, tannins and cyanogenetic glycosides. Alkaloids and anthraquinones were not present [44]. The root, stem bark and leaves aqueous and methanolic extracts of X. americana were tested against five bacteria and they inhibited the growth of *Staphylococus aureus* and *Klebsiella pneumonia* while *Shigella flexineri* was inhibited by only methanolic leaves, aqueous bark and aqueous leaves extracts. *Salmonella typhi* and Escherichia coli were not affected by these extracts. The Minimum Inhibitory Concentration (MIC) was only evident for the methanolic extracts at 1.25x104 µgmL-1 (1:4) against *Staphylococus aureus* while the Minimum Bactericidal Concentration (MBC) of the extracts was obtained at 2.50x10-4µg mL-1 (1:2) [42]. From the results, inhibitory activity of extracts (methanolic root) was more pronounced

on *Klebsiella pneumonia* whereas it shows no activity against *Escherichia coli*, Salmonella typhi and *Shigella flexineri*. The methanolic root extract showed highly significant ($p<0.05$) activity on *Klebsiella pneumonia* when compared with leaf extracts and methanolic bark extract. The phytochemical constituents present in the extracts were carbohydrates in the form of sugars and soluble starch (except for aqueous and leaves extracts), cardiac glycosides, saponins, tannins and flavonoids while alkaloids were absent in all the extracts. It was concluded that the extracts of methanolic roots, stem bark and leaves have bacteridal activities over the concentration of 2,5x104 - 1,25x104 µgmL-1 and that the presence of carbohydrates, glycosides, flavonoids and tannins in the diferent extracts are responsible for their antibacterial activity. The antimicrobial properties of the bark, leave, root and stem extracts of Ximenia americana were screened against Bacillus subtilis, *Staphyllococus aureus*, *Escherishia coli* and *Pseudomonas aeruginosa* using the cup-plate agar diffusion method and the minimum inhibitory concentration by agar dilution method [47].

In summary, the results justified the use of X. americana as having antibacterial properties and support its use as agent in new drugs for therapy of infectious diseases caused by pathogens.

2.2.2 Pesticidal activity:

Olecaceous seed oils are a rich source of acetylenic lipids and unsaturated fatty acids [48, 49]. Acetylenic metabolites show some biological activities including, insecticidal activity [50]. X. americana was recorded to contain octadec-11-en-9-ynoic acid, named xymeninic acid as well as icosenoic-triacontenoic acids, all of which belong to the ω-9 series [51]. Bioactivity-driven fractionation of the CHCl3 extract of the root of X. americana using the Brine Shrimp Lethality Test (BST) and hatchability test with Clavigralla tomentosicollis eggs yielded two fractions (F006, soluble in petroleum ether and F005, soluble in 10% H2O in MeOH) as the most actives (F005, BST LC50 78 (129-48) µg/mL and F006, BST LC%50 76(121-49) µg/mL) [52]. A combination of F005 and F006 was submitted to hatchability test (inhibition of hatching = 68 % of control) and successive BST-dircted fractionation on silica gel column and preparative TLC yielded oleanene palmitates (1), β-sitosterol (2) and C18acetylenic fatty acids (3 and 4) as yellow oils.

Phytochemicals – A Global Perspective of Their Role in Nutrition and Health 436 mol wt 6 mass units less than that of 3 with molecular formula C18H26O2 as revealed by HREI-MS (m/z 274.2021 for [M+]) in combination with its 1H and 13CNMR spectra. The 13C NMR spectrum of 4 displayed absorptions diagnostic of acetylenic carbons at δC 83.4 (C) and 74.1 (C) and of carboxylic carbon at 179.3 (C), in agreement with its IR spectrum which exhibited bands at 2232 and 1702 cm-1, characteristic of acetylenic and acid groups, respectively. The 13C RMN spectrum also exhibited six resonances at δC 148.2 (CH), 140.9 (CH), 136.9 (CH), 129.8 (CH), 109.3 3(CH) and 108.6 (CH), revealing the presence of three double bonds. From a detailed spectral analysis considering, especially, the multiplicity of signals and coupling constants in the 1H NMR spectrum, as well as the presence of diagnostic peaks in the mass spectrum, compound 4 was thus established as 10Z,14E,16E-octadeca -10,14,16-triene-12-ynoic acid, a ene-ene-yneene acetylenic fatty acid [52]. In addition, Ximenia seed oil have been found to contain fatty acids with more than 22 carbon atoms (very long fatty acids) which are found only rarely in nature. Using liquid chromatography in combination with mass spectrometry was founded that Ximenia oil to contain fatty acids with chain length C34 and C36 [51]. Effectively, two very long chain unsaturated fatty acids C40 and C35 (5 and 6) were isolated [53] from X. americana seeds and fruits, respectively. The mass spectrum of the major component (5) showed a molecular ion at m/z 604 corresponding to the molecular formula C40H76O3. The IR spectrum of 5 showed a broad absorption band at 3600-3200 cm-1 (OH) and the presence of strong absorption at 1742 cm-1 attributed to ester group. The base peak appeared at m/z 55 (C4H7+) due to allylic bond cleavage and peaks at m/z 479 and 151 furnished from fragmentation in C28-C29 and C26-C27, respectively. In addition, the peaks at m/z 31, 59, 73 and 74 (McLafferty rearrangement) were compatible with unit CH3OCO(CH2)3-. The compound 5 was identified as methyl-14,14-dimethyl-18-hydroxyheptatracont-27,35-dienoate. The mass spectrum of 6 showed a molecular ion at 578, corresponding to the molecular formula C35H62O6. The IR spectrum showed bands at 3500, 1731 and 1645 cm-1 corresponding to OH, C=O and C=C groups, respectively. The base peak appeared at m/z 73 (C3H5O2+) which is characteristic for the methyl ester, reinforced by additional peaks at m/z 31, 59 and 74 (McLafferty rearrangement). A peak at m/z 479 was due to M-C5H7O2 and one at m/z 339 is due to the cleavage C13-C14 while, those at m/z 126 and 265 were due to C7H10O2 and M-C17H28O2, respectively. The compound 6 was identified as dimethyl-5-Methyl-28,29-dihydroxydotriacont-3,14,26-triendioate.

2.2.3 Analgesic activity:

The aqueous extract of stem bark of X. american has analgesic properties that justify its use popular in countries such as Tanzania, Senegal, Zimbawe and Nigeria. The extract of X. americana in doses containing 10 to 100 mg/kg P.C, inhibits contractions of the abdomen with analgesic effects comparable to those of phenylbutazone. In fact, at doses of 100 mg / kg P.C, phenylbutazone causes an inhibition of pain in 45.2±2%. The percentage of inhibition by extract of X. ameriacana is 61.1±% in the same concentration. These properties are probably due to the presence of flavonoids and saponins, detected in the extract [54]. The analgesic activity of the methanol extract of X. americana leaf was investigated in chemical models of nociception in mice. The extract at doses of 200, 400 and 600 mg/kg i.p. produced an

inhibition of 54.13, 63.74, and 66.4% respectively, of the abdominal writhes induced by acetic acid in mice. In the formalin test, the administration of 200, 400 and 600 mg/kg i.p. had no effects in the first phase (0 to 5 min) but produced a dose dependent analgesic effect on the second phase (15 to 40 min) with inhibitions of the licking time of 29.3, 47.8 and 59.8%, respectively. These observations suggested that methanol extract of X. americana leaf possesses analgesic activity [55].

2.2.4 Antipyretic activity:

The bark of stem of X. americana has been used in West Africa for the treatment of pain and fever. To verify this second property, the treatment of rats in hyperthermia with Ximenia americana stem bark aqueous and with beer yeast was compared to those obtained with lysine acetylsalicylate (Aspegic). The study showed an antipyretic action of the extract. Moreover, the toxicological study of the stem extract indicated a LD50 of 237.5 mg/kg P.C according to the classification of Diezi this plant is relatively toxic. The experiments show that the properties of X. americana could due to the presence of saponosides, as shown by screening tests performed in this study. These results justified the use of X. americana in traditional cure of fever treatment [54].

2.2.5 Antitrypanosomal activity:

The in vitro antitrypanosomal activity of methanolic and aqueous extracts of stem bark of Ximenia americana was evaluated on Trypanosoma congolense. Blood obtained from a high infected mice with T. congolense was incubated with methanolic and aqueous extracts at 20, 10 and 5 mg/ml and Diminal (R) (diminazene aceturate) at 200, 100 and 50 µg/ml in a 96 micro plate. The results revealed that methanol and aqueous extracts had activity at 20 and 40 mg/ml however, the methanolic extracts were more active than aqueous extracts at 10 and 5 mg/ml. Phytochemical screening of the methanolic and aqueous extracts of the bark showed that they both had flavonoids, anthraquinones, saponins, terpenes and tannins. The aqueous and methanolic extracts appears to show some potential activity against T. congolense [36].

2.2.6 Anticancer activity:

Plants have been shown to provide a useful source of natural products that are effective in the treatment of human neoplastic diseases. Information recorded from ancient civilizations has demonstrated the use of plants in search of treatment for various types of cancer [56]. An analysis of plant materials that had been studied at the National Cancer Institute (NCI), USA for discovering new anticancer drugs showed that if ethnopharmacological information had been used, the yield of plants harboring antineoplastic activity would have been significantly increased [57]. The list of natural products stored for study as more effective drugs for the treatment of human cancers (NCI) were generated by searching for specific structural types [58]. However, the presence of some large class cannot be ruled out. Examples of anticancer agents developed from higher plants are the antileukemic bis-indole alkaloids vinblastine and vincristine from the Catharantus roseus (Apocynaceae); diterpene taxol, used to treat breast cancer, lung cancer, and ovarian cancer and also used to treat AIDS-related (Kaposi's sarcoma) from Taxus breviflora (Taxaceae); pyrrolo[3,4,b]-quinoline alkaloid camptothecin (antileukemic) from Camptotheca acuminate (Nyssaceae) and pyridocarbazole alkaloid elipticine (antitumor) contained in Ochrosia elliptica (Apocynaceae). A large number of other active natural products with toxicity to cells in culture (Walker carcinosarcoma 256, mouse L-1210 leukemia, Ehrlich ascite tumor, sarcoma 180 and mouse P-388 leukemia cell lines) have been detected [59].

2.2.7 Antiviral effect:

The stem bark MeOH extract of X. americana as well as several others plant species used by the Maasai pastoralis of East Africa showed antiviral effect against measles virus in vitro by plaque reduction neutralization assay. Potentially active constituents from extracts of all the plants include polyphenols, alkaloids, tannins, sterols, terpenes, saponins and glycosides, between others [60].

2.2.8 Hepatic and heamatological effects:

A study was conducted from the leaves, stem bark and root aqueous extract of X. americana with albino rats [61]. The results of this work shows that the extracts significantly ($P<0.05$) increasing the level of serum alanine transaminase (ALT) and aspartate transaminase (AST), results indicative of hepatocellular damage. The result also shows that the root has the ability to impair albumin synthesis as observed by the decrease of level of serum albumin. The weight of the animal showed a significant ($P<0.05$) reduction on administering the leaves extract as compared to the control and the others extracts. This reduction might be due to poor intake and utilization of food by the animals in the leaves extract group. The significantly ($P<0.05$) higher content of hydrogen cyanide, saponins, and oxalates in the root extracts indicates that the root extracts may be more toxic. Hydrogen cyanide is known to cause gastrointestinal inflammation and inhibition of cellular respiration. Saponins are known to have haemolytic properties and the ability to reduce body cholesterol by preventing its reabsorption. The high saponin content in the root may lead to gastroenteritis manifested by diarrhea. Oxalates have been known to cause irreversible oxalate nefrosis when ingested in large doses. Thus, there is need to isolate the specific component(s) responsible for the toxicity in the root extract in order to standardized the preparation for maximum therapeutic benefit.

2.2.8 Toxicity:
The stem bark of X. americana was evaluated for its phytochemical constituents and acute toxicity effect on the Swiss albino mice [36]. The results from the extracts administered intraperitoneally/orally at doses of 10, 100 and 1000 mg/kg body weight revealed no death with doses up 5000 mg/kg body weight. Post mortem, hematological and histopathological examination did not show any significant ($P<0.05$) weight changes. Phytochemical screening of the aqueous extract stem bark revealed the presence of cardiac glycosides, flavonoids, saponins and tannins. The results suggested that the aqueous extract is not acutely toxic to the mice.

2.2.9 Food composition and cosmetic use:
Glyceride blends containing ximenynic acid (found in X. americana) are useful for the preparation of food compositions or food supplements, including margarine, chocolate, ice cream, mayonnaises, cheese, dry soups, drinks, cereal bars and sauces and snack bars. The blend provides a composition providing health benefits consisting of insulin resistance, or related disorders such as diabetes, delaying the onset of symptoms related to development of Alzheimer's disease, improving memory function, lowering blood lipid levels, anticancer effects or skin antiageing effects [62]. Food X. americana flowers are a replacement for orange blossoms with similar fragrance and soothing cosmetic properties [63].

Previous studies from our laboratories also showed aqueous and methanolic *Ximenia americana* leaf extracts were potent antioxidant and anti-inflammatory agent [64]. Hence, the present study also supporting and provides a clear scientific basis that *X. americana* can be a potent source for the novel medicines. Antidiabetic activities of aqueous *X. americana* leaves extract reported for the first time showed its therapeutic potential to be used as a cost-effective safe herbal antidiabetic agent [65]. Accordingly, these results encourage further studies on extracts and identify particular active chemical compounds responsible for the specific biological activity in order to standardize the plant preparation for maximum therapeutic benefit to treat diseases.

The extensive literature survey exposed that only few reports exist on this plant seeds and leaves, but no information are available on other uses activity in particular as antioxidant. Henceforth, our study was goal to explore the phytochemical constituents of *Ximenia americana* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis and also evaluating the antioxidant activity of the different solvent extracts.

3. MATERIALS AND METHODS

3.1 Collection and identification of plant materials:
A fresh sample of the plant was collected from west Sudan (Kordofan). The fruits of *X. americana* were collected from forest around Babanosa during rainy season August. The fruits were first identified by a traditional healer, after which they were further identified, confirmed and authenticated at the Department of pharmaceutical Sciences in Al Gezira University.

3.1.1 Extraction of seed oil from *X. americana:*
The fresh fruit of Ximenia Americana were freed from the coating layer for obtaining seed, and seeds after drying at room temperature for one week were reduced in small granules and must be crushed to coarse powder. A sample (100g) of coarse powder seeds was extracted by maceration in conical flask for 48 hours used a 500ml of ethanol and continuously shaking. The filtered extract was evapourated under vacuum at 60^{O}C using a rotary evaporator. The oil obtained (52.2%) was kept at room temperature in a sealed vial.

3.1.2 Sample preparation for Gas Chromatography-Mass Spectrometry:
To 5 ml of oil, 20 ml of methanol and 10 ml of benzene were added. Concentrated H_2SO_4 (1.0 ml) was added to the mixture and was then shaken. The reaction mixture was refluxed on a water bath at 50 ˚c for 90min, with intermittent shaking (Harbone 1984). The mixture was allowed to cool and was shaken twice with 20 ml petroleum ether. The petroleum ether was removed was removed by evaporation on water bath at 50 ˚C to give the methylated oil fraction.

3.2 GC/MS condition:
The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japans Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30×0.25 mm×0.25µm). The sample was injected by using spilt model, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60 ˚c with rate 10 c/min to 300˚c as final temperature degree with 5 minute hold time, the injection port temperature was 300˚c, the ion source temperature was 200 ˚c and the interface temperature was 250˚c. The sample was analyzed by using scan mode in the range of m/z 40-500 charge to ratio and the total run time was 29 minutes. Identification of component for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST).

3.3 Moisture content (%) in grains of *X. Americana:*

The grains (30.974g) were oven-dried at a temperature of 105°C for 24h, time required to obtain a constant mass (21.394g). The moisture content was obtained according to the equation below, with a value of 31.32%.

$$\text{Moisture (\%)} = \frac{mi - mf}{mi} \times 100$$

At where:

mi = initial seed mass.

mf = final seed mass.

3.4 Oil content in (%) in grains of *X. americana:*

The oil content was determined from the internal part of the grains (dehydrated matter = 21.394g) using a Soxhlet type extractor and hexane as solvent in a continuous and uninterrupted extraction process for 6:00 h. After this time, the solvent was evaporated under reduced pressure to give a mass of fixed oil (5.90 g), which represented 27.71% oil in the beans.

3.5 Characterization of Ximenia americana Oil:

3.5.1 Refractive index:

The refractive index of substance is the ratio of the speed of light at definite wavelength in vacuo to its speed in the medium.

In practice the speed of light in air is taken in the place of the speed in vacuo. Unless otherwise stated, the selected wavelength is mean wavelength of the D-line od sodium(589.6nm).

The refractive Index is determined at 20 º C for liquid oils which are completely liquid at this temperature, but its 40 º C for palm olein, 50 º C for palm oil 60 º C palm stearin and 80 º C for hydrogenated fats.

The notation for refractive index t d where it is temperature of the measurement in ºC

Principle:

Measurement by means of suitable refractometer of refractive index of the oil sample

Scope:

Applicable to normal oil and fats

Reagents:

1. Petroleum ether (60 – 80 º C) is satisfactory for cleaning the prisms

Apparatus

1 Refractometer e.g . Abbe type with scale graduated in refractive index units, and able to measure the refractive index to ± 0.0001 within the range n D = 1.3000 to 1.7000.

2 Light source: sodium vapour. A tungsten lamp can be used if the refractometer is equipped with an achromatic compensating device.

3 Water- bath, thermostatically controlling with circulation pump to maintain the temperature of the refractometer to within ± 0.1 º C.

Preparation of sample for analysis:

Melt the sample at 10 º C above the melting point of the sample and homogenize before sampling. The sample must be clear. Filter through a dry filter paper if necessary.

Procedure:

1. Prepare the refractometer and calibrate it. Adjust the temperature of the circulating liquid from the water bath to the required temperature noted.
2. Place a few drops of the sample between the prisms of the refractometer in such a way that the space between the prisms is completely filled. Wait for 2 – 3 mins to allow the fat to assume the temperature of the prisms.
3. Read the refractive index, estimating to tenths of a scale division (0.0001).
4. Carry out two determinations on the same sample prepared.

Expression of results:

Take as the result the arithmetic mean of two results, corrected if necessary if the requirement concerning repeatability (see 9) is satisfied. Round to the fourth decimal place.

Repeatability:

The difference between the results of two determinations carried out in rapid succession by the same analyst should not exceed 0.0002.

3.5.2 Determination of Colour:

The Colour of sample is the Colour in Lovibond units as measured on a Lovibond Tintometer.

Pripciple:
The method consists of matching the colour of the light transmitted through a specified depth of oil to the colour light, originating from the same source transmitted through standard Colour slides.
Scope:
Applicable to oils and fats.
Apparatus:
1. Lovibond tintometer, equipped with standard Colour slides
2 . Glass cells, with lenqths of light path 1inch (25.4mm) and 51/4inch (133.4mm)
Selection of operators:
All operators shall satisfy the requirement of colour vision test and shall be retested of five years intervals.
Tinted or light spectacles or lenses shall not be worn.
The tintometer shall be maintained in good clean condition in accordance with the manufacture's recommendations lighting cabinet, consisting of two 60W pearl (no coated) lamps, operated at the correct mains voltage and each illuminating the sample white reference field at 45 c̊.
The lamps shall be positioned at either side of the viewing tube.

The lamps shall not be used for longer than 100hrs, as suitable logging system shall be employed to register lamp life. Lamps shall be changed in pairs.

The viewing tube shall have a field of view of subtending 4c̊ to the eye and contain a daylight correction filter.

The lighting cabined shall be such that it enables the sample and white reference field to be viewed at 90 c̊.

The lighting cabinet shall be inspected of frequent interest for any dirt articles, which shell be removed and for equaling of the paint. The cabinet shall be repainted with a matt white point when the colour becomes darker than Man self-notation 5Y9/1 (obtainable from the tintometer) Colour racks, consisting of red, yellow, blue and neutral racks with colour readings as follows, and fitted with colour less compensating slides.
Red 0.1to 0.90; 1.0 to 9.0; 10.0and 20.0
Yellow 0.1to 0.90; 1.0 to 9.0; 10.0and 70.0
Blue 0.1to 0.90;
Neutral 0.1to 0.90; 1.0 to 3.0;
Procedure:
1. If prepared sample is not liquid a room temperature heats it to a temperature of 60 ± 5 c̊. And pour into the cell. The sample should be clear and bright when determination is made.

For crnde and bleached oils, choose a 1inch(25.4mm) cell. for refined oils a 51/4 inch (133.4 mm) cell is suitable.

2. using the lovibond tintometer, immediately determine the colour of the sample by achieving the best possible match with standard Colour slides.

Since the onset of eye fatigue is rapid (i.e. after about 10 seconds) it is preferable, if acolour match cannot be quickly obtained, to repeat the procedure after a few minutes. it is also desivable that the test be carried out by trained operators. If the red, yellow, blue and neutral unit's do not form possible match of the colour of the oil, use a minimum of blue units to obtain the suitable match.
Expression:
Express the results in terms of:
A. The number of red, yellow and neutral or blue units needed to obtain the match and B. The cell used and the lovibond model number.
Reproducibility:
The difference between the results obtained by two trained operators carrying out the procedure described using the same apparatus in the same laboratory, simultaneously or in rapid succession shall not exceed the values given in table 1

Table 1: Acceptable Colour Reading differences

Reading	Acceptable differences
Up to 0.9 Red	0.2 Red
From 1.0 Red to 2.9 Red	0.4 Red
From 3.0 Red to 4.0 Red	0.5 Red
From 4.1 Red to 12 Red	1.0 Red

3.5.3 Determination of Density:
Density was determined by the pycnometer method using a 25 mL glass pycnometer. The density values were determined at four different temperatures and they are the mean of three independent determinations.

3.5.4 Determination of Acid Value:

The acid value is the number of milligrams of potassium hydroxide necessary to nutralise the free acid in 1 g of sample. The acidity is content of free fatty acid conventionally expressed as percentage (m/m) of palmatic acid.

Principle:

A know mass of the fat is dissolved in neutralized isopropanol the free fatty
acids are neutralized with standard alkali.

Scope:

Applicable to all normal oils and fats.

Reagents:

1. Standard potassium or sodium hydroxide 0.1 N, for crude oils, and 0.02 N for refined oils.
2. Phenolphthalein indicator solution, 1.0% in isopropanol (Ethanol 95% v/v may also be used).
3. Neutralized isopropanol (Ethanol 95 % v/v may also be used). Place 50ml isopropanol or Ethanol 95% v/v in a flask and bring the solution to boil over a hot plate. Add about 0.5 ml of phenolphthalein indicator and neutralized by drop wise addition of 0.1 potassium or sodium hydroxide till faint, but permanent pink color obtained.

Apparatus:

1. Burette, 25 ml with graduation in 0.05 ml division
2. Conical flask of 250 ml and 500 ml.
3.Hot plate with temperature control.
4. Analytical balance.

Preparation of sample for analysis:

If the sample is not liquid melt at 60 – 70 º C and thoroughly homogenize it before sampling.

Procedure:

Weight the specified amount of sample into an Erlenmeyer flask. Add the neutralized solvent as in the table above.
Place the flask on the hot plate and regulate the temperature to about 40ºC. Shake the sample gently while titration with standard alkali to the first permanent pink colour. The colour must persist for 30 seconds.

Expression of results:

FFA% as palmatic acid = $\frac{25.6 \times N \times V}{W}$
(for palm oil and fraction)

FFA% as Lauric acid = $\frac{20.0 \times N \times V}{W}$
(for coconut oil, palm kernel oil and fractions)

FFA% as Oleic acid = $\frac{28.2 \times N \times V}{W}$
(for corn oil, soybean oil And other liquid oils)

Where :
N: normality of NaOH solution.
V: volume of NaOH solution used.
W: weight of sample.
Express the FFA to 3 decimal places for FFA below 0.15% and 2 decimal places for FFA above 0.15%.

Repeatability:

The difference between the results of two determinations on the same test sample, carried out simultaneously or in rapid succession by the same analyst should not exceed 0.02% for free fatty acid contents between 1.5% and 5.0% and 0.004% for free fatty acid contents less than 0.1% (AOCS 5a – 40 (1989).

3.5.5 Percentage of Free Fatty Acid (FFA):

The percentage of FFA (as oleic acid) was obtained multiplying the acid value by the factor 0.503: %FFA = IA × 0.503.

3.5.6 Determination of Saponification Value:

The saponification value Sv (mg KOH/g oil) was determined according to the A.O.A.C., 17th Ed, 2000, Official method 920.160, as is described in Manual of Methods of Analysis of Foods.

The saponificaion value is the number of milligrams of potassium hydroxide required to saponifity 1 g of fat under the conditions specified. It is a measure of the average molecular weight of all the fatty acids present.

Principle

The glycerids present are split by alcoholic alkali and any free fatty acids are neutralized. Excess alkali is back – titration with hydrochloric acid in the presence of an indicator.

Scope:

Applicable to all normal oils and fats.

Reagents:

1. Potassium hydroxide, approximately 0.5 N solution in 95% (v/v) ethanol.

2. The solution should be colourless or straw yellow.
3. Phenolphthalein solution 1% (v/v) in 95% ethanol.
4. Boiling aids e.g. pumice stone.

Apparatus:

1. Conical flask, 250 ml capacity, made of alkali resistant glass, with ground neck.
2. Reflex condenser, with ground glass joint to fit the flask.
3. Water bath, or hot plate with variable heat control.
4. Burette, 50 ml capacity graduated in 0.1 ml divisions.
5. Pipette 25 ml.

Preparation of sample:

Melt and homogenize the sample if it's not liquid at 60 – 70º C before taking
a test portion. Filter through a dry filter paper to remove any impurities.

***Procedure*:**

- Weight to the nearest 0.005 g, a test portion in to a conical flask such that the volume of hydrochloric acid required for the determination will about half that for the blank determination. This weight will usually be around 2 g.
- Add 25 ml of ethanolic potassium hydroxide solution and some boiling aids. Connect the reflux condenser and boil gently at least 60 minutes, swirling, the content of the flask from time to time.
- Allow the flask and condenser to cool slightly before washing the inside of the condenser with a little distilled water. Add 1 ml of phenolphthalein solution and titrate with 0.5 N hydrochloric acid until the pink colour of the indicator just disappears.
- Conduct a blank determination simultaneously with the test sample using the same procedure Expression of results: The saponifaible value is given by:

$$\mathbf{SV = 56.1\ N\ (V\ b – V\ s)\ W}$$

Where :
V b : is the volume, in milliliter of the hydrochloric acid used for the blank.
V s : is the volume, in milliliter of the hydrochloric acid used for determination of the sample.
N: is the normality of the hydrochloric acid.
W: is the weight, in grams of the test portion.
Express the result to the nearest unit

***Repeatability*:**

The difference between the result of two determination on the same test sample, carried out simultaneously or in rapid succession by the same analyst, should not exceed 0.5 % of their main value.

3.5.7 Determination of Iodine Value:

The iodine value (g I_2/g oil). The Iodine value is a measure of the unsaturation of fats and oils. It is expressed as the number of grams of iodine absorbed by 100 g of the fat under the test conditions used.

Principle:

Addition of a solution of iodine monochloride in a mixture of acetic acid and carbon tetrachloride to test portion. Reduction of the excess of iodine monochloride after a specified reaction time by adding potassium iodide solution and water, and titrate of the liberated iodine with standard sodium thiosulphate solution.

Scope:

Applicable to all fats and oils that do not contain conjugated double bonds.
Applicable to all normal fats and oils with samples having iodine values between 0 – 15

Reagents:

1. Carbon tetrachloride, free from oxidizable matter.
2. Glacial acetic acid, free from ethanol and oxidizable matter.
3. potassium iodide 10% (w/v) aqueous solution, free from iodine or iodate.
4. Starch solution 1% (w/v) aqueous dispersion, freshly boiled for 3 minutes and allow to cool.
5. Sodium thiosulphate 0.1 N, dissolve 24.8 g of sodium thiosulphate pentahydrate in distilled water and dilute to 1 litre.
6. Potassium dichromate, 0.1 N. Dissolve 4.9035 of dried potassium dichromate in distilled water and make up to 1 litre.
7. Wijs reagent (Iodine monochloride solution in acetic acid, carbon tetrachloride mixture). The reagent is available commercially. It may be prepared from iodine trichloride as follows:

Weight 9 g of iodine trichloride into a brown glass bottle 1500ml capacity.
Dissolve in a mixture of 700ml glacial acetic acid and 300ml carbon tetrachloride.
Take 5ml of the solution and add 5ml of potassium iodide solution and 30 ml of water. Titrate the liberated iodine with 0.1N sodium thiosulphate solution in the presence of a few drops of starch indicator.

Add 10g of pure re – sublimed iodine to the bulk of the reagent and dissolve by shaking. Titrate the free iodine as above, the titrate should now equal one and a half time that of the first determination. If this is not the case, add small quantity of

iodine until the content slightly exceeds the limit of one and half times. It is important that no trace whatever of iodine trichloride should remain as it would cause secondary reaction. Let the solution stand. Decant the clear liquid into a brown glass bottle. If stored in a well – Stoppard bottle away from light, the solution can be used for months.

Apparatus:

1. Glass weighting scoops or vail of 2 – 4 ml capacity for test portion.
2. Wide – necked glass bottle or flask with ground Stoppers (e.g. iodine flasks) capacity 250ml or 500ml.
3. Burette, 50ml graduated in 0.1ml.
4. Pipette, 20 and 25 ml.

Preparation of sample for analysis:

Melt the sample at 60 – 70° C (If it is not liquid) and thoroughly homogenize before taking test portion.

Procedure:

- Weight the sample accurately to the nearest 0.0001 g in the glass weighting scoop or vail
- The weight of the sample to be used varies according to its excepected iodine value. Use the table below as a guide.
- Place the scoop in 500 ml flask. Add 15 ml of carbon tetrachloride to dissolve the fat. If necessary, warm the flask slightly to facilitate dissolution of the fat before addition of carbon tetrachloride solution. Add exactly 25ml of the wijs solution, insert the stopper, shake gently and place the bottle in the dark for 1 hour.
- After standing add 20 ml of potassium iodide solution and 150 ml of water.
- Titrate with the sodium thiosulphate solution until the yellow colour due to iodine has almost disappeared. Add 1 to 2 ml of starch indicator solution and continue the titration until the blue colour just disappears after very vigorous shaking.
- Carry out two determinations on the sample test under the same conditions.

Expression of Results:

$$\text{Iodine Value} = \frac{\mathbf{12.69 \times N (V\,2 - V\,1)}}{\mathbf{W}}$$

Where:

N : IS the exact normality of the sodium thiosulphate solution used,

V 2 : is the volume in mille liters, of the sodium thiosulphate used for blank test.

V 1 : is the volume in millie liters, of the sodium thiosulphate used for determination.

W : is the weight in grams, of the test portion.

Express the result to one decimal place

Repeatability:

The difference between the result of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0.5 unit of iodine value.

3.5.8 Determination of Peroxide Value:

The peroxide value (mg O_2/kg oil).

The peroxide value is the measure of those substances in a sample, expressed in terms of milliequivalents of active oxygen per kilogram which oxidize potassium iodide under the conditions of test.

Detection of peroxide gives the initial evidence of rancidity in saturated fats and oils. Other methods are available but peroxide value is most widely used. It gives a measure of the extent to which and oil sample has undergone primary oxidation, extent of secondary oxidation may be determine from p – anisidine test. The double bond found in fats and oils play a role in autoxidation (oils with high degree of unsaturation are most suspectable to autoxidation) which is a free radical reaction involving oxygen that leads to determination of fats and oils which for off – flavors and off- odours.

Principle:

Treatment of a test portion in a solution of acetic acid and chloroform with a solution of potassium iodide. The free iodine is titrated against a solution of sodium thiosulphate.

Scope:

Applicable to all normal oils and fats.

Reagents:

All reagents and water shall be free from dissolved oxygen (This is can be achieved by passing a stream of pure, dry inert gas (carbon dioxide or nitrogen) through individual reagents.

1. Acetic acid, chloroform solution mix three parts by volume of glacial acetic acid with two parts by volume of chloroform.
2. Potassium iodide, a saturated solution prepared in recently boiled water. The solution must remain saturated.
3. Sodium thiosulphate (Na 2 S 2 O 3 .5H 2 O), 0.01 N standard volumetric solution, standardized before use.
4. Starch solution Mix 5 gm of soluble starch in 30ml of water add this mixture to 1000 ml of boiling water and leave boiling for three min.

Apparatus:

1. Pipette 1 ml, graduated in 0.01 ml division.
2. Conical flask, glass stopper, 250 ml.
3. Volumetric flask, 500 ml, 100 ml.
4. Burette 25 ml, graduated in 0.05 ml division.

Sample preparation:

All samples should be analyzed as soon as possible or should kept in a cool, dark place before analysis.

Procedure:

Weight to nearest 0.1 mg, 5.00 ± 0.05 g.

Add 30 ml of acetic acid – chloroform solution swirl the flask until the sample is dissolved in the solution add 0.5 ml of saturated potassium iodide with a graduated pipette. swirl solution for 1 min and then add 20 ml of distilled water. For freshly produced oils, add a few drops of starch solution. Titrate with 0.01 N sodium thiosulphate solution. Adding it gradually and with constant and vigorous shaking. Continue the titration, shaking the flask vigorously near the end – point to liberate all the iodine from the chloroform layers. Add the thiosulphate solution drop wise until the blue colour just disappears.

From samples with high peroxides, titrate with 0.01 N until the yellow iodine colour has almost disappeared. Add 0.5ml of starch indicator and continue titration until the blue colour just disappears.

Carry out a blank test in parallel with the determination. The blank titration must not exceed 0.1ml of the 0.1N sodium thiosulphate solution.

Expression of results:

The peroxide value, expressed in mill equivalents of active oxygen per kilogram of sample is

$$\frac{(V_s - V_b)\, N \times 1000}{W}$$

Where

V s : is the volume in milliliters of sodium thiosulphate solution of normality N, used for the determination.

V b : is the volume, in milliliters, of the sodium thiosulphate solution used for the blank test.

W : is the weight, in grams, of the test portion.

N : is the normality of sodium thiosulphate solution.

Express the result to one decimal place.

Repeatability:

The difference between the results of two determination carried out simultaneously or in rapid succession by the same operator on the same sample shall not exceed the following:

Peroxide value (meq/kg)	Repeatability
Less than 1	**0.1**
1-6	**0.2**
6-12	**0.5**
Greater than 12	**1.0**

3.5.9 Determination of Viscosity:

The viscosity of samples was measured with a TA Instruments AR1500ex rheometer using plate/plate geometry for selected temperatures relevant for the intended use. The temperature (30 °C) is controlled by a Peltier system existing on the lower 100 mm diameter stainless steel plate with a 0.01 K resolution, the thermometer being located adjacent to the center of the plate just below the sample layer. The upper geometry used for all samples was a 40 mm diameter stainless steel plate.

The sample volume required for the selected gap (about 1000 µm) was loaded on the geometrical center of the lower plate and the upper plate was sent to the grim gap (selected gap + 200 µm). The final gap was adjusted to meet the proper filling of the geometry.

3.5.10 Evaluation of UV Absortion:

UV spectra of the three oils were obtained by deposition of a thin layer (0.6 mg/cm^2) over a quartz plate. Spectra were acquired in a Shimadzu UV 1603 spectrophotometer with the cell compartment the rmostatized at 35 °C.

3.5.11 Chemical Composition:

The chemical composition of the oils was determined by GC-MS after derivatization of the triacylglycerides in the corresponding methyl esters.

3.5.12 Determination of cis – and trans – fatty acids in hydrogenated and refined oils and fats by capillary GLC:

This method consists of gas – liquid chromatography (GLC) conditions to identify and quantity the trans fatty acids isomers in vegetable oil and fats. The fatty acid methyl Estes (FAME) of the sample are separated on capillary gas chromatography column having a high polar stationary phase, according to their chain length (CL), degree of (un) saturetion caturation, and geometry and position of the double bond (DBs).

Scope:

This method is especially designed to evaluate, by using capillary GLC procedure, the level of trans isomers as formed during (high – temperature) refining or during hydrogenation of vegetable oils or fats.

The method may also be used to report all other fatty acids, for example to obtain saturated fatty acid (SAFA), monosaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), LEVELS FROM THE SAME SAMPLE ANALYSIS.

Apparatus:

1. Gas chromatography – equipped with capillary injection system (preferably split mode, operated at spilt ratio of approximately 1: 100) and flame ionization detector (FID), capable of meeting the following requirements:
2. Injection port temperature, 250° C, detector temperature, 250° C, oven temperature conditions.
3. Column – high polar stationary phase, such as one of the following:

(a) CP™ – Sil 88, 100 or 50 mx 0.25 mm i.d., 0.20 um film (chrompack, Middleburge, The Nethelands)
(b) SP – 2650, 100 m X 0.25 mm i.d, 0.20 um film (supleco Inc, Belleford, PA, USA)
(c) SP – 2340, 60 X 0.25 mm i.d, 0.20 mm film (Suplec Inc).
(d) BPX – 70 ,120 or 120 m X0. 22mmi.d, 0.25 mm film (SGE Inc, Austin, TX, USA).
(e) SP 2560 (50TO 100) m X 0.25 mm i.d, 0.20 mm film.

3. Recording instrument.

Reagents:

Use reagent of recognized analytical grade and water of at least grade 3 as defined in ISO 3696.

1. Carrier gas – helium, nitrogen, or hydrogen, GC quality, dried and oxygen removed by suitable filters.
2. Internal standard (for calculating fatty acid data as per mg oil) – tridecanoin , 5.0 mg /ml in chloroform. This solution is stable up to 1 week if stored in refrigerator in well-sealed amber bottle.

Proposed optimal GLC conditions for identification of trans isomers in refined and hydrogenated vegetable oil samples.

Stationary phase SP- 2560 SP- 2560 SP- 2560 tem. conditions Isothermal 192° C, Isothermal 170° C Isotherm 175°, Isotherm 198° C column heat pressure (kpa) 125.

Stationary phase	**SP-2340**	**SP-2560**	**SP-2560**	**SP-2560**
temp. conditions	Isothermal 192° C	Isothermal 170° C	Isothermal 175° C	Isothermal 198° C
column heat pressure (kpa) 125	125	125	130	155
Linear velocity of carrier gas (He)	125 cm/sec	16 cm/sec	19 cm/sec	17 cm/sec

Procedure:

Sample preparation:

(a) Prepare the methyl esters from triglycerides from the oil or fats to be analyzed, using boron trifluoride method as it described, for example in AOCS official method Ce 2– 66 or IUPAC 2.301.

(b) Before test portion are taken from sample, the sample should be mixed thoroughly. Solid samples should be melted to insure proper mixing.

Chromatography:

(a) Set up gas chromatography with the temperature and column as described in table 1. Measure the average a carrier gas linear velocity as indicated in table 1, with split ratio of approximately 1:100.

(b) Injection 0.5 to 1 ul /l of the methyl Easters (concentration approximately 7 mg/ml) from the test sample in to the gas chromatograph.

Compare the result with the example chromatograms (Figure 1 to 5). If the separation obtained is not identical to the example chromatograms, small changes in oven temperature may require. If so, decrease or increase the oven temperature with subsequent step of 1 C until a good separation is obtained. These small corrections might be required to correct for batch differences between column and instrument temperature and generally all within a range of only a few degrees (plus or minus) at maximum from the indicated value. The 20: 1 c peak will elute earlier relative to 18:3 ccc if the oven temperature is increased.

Performance check:

(a) If the GLC system is set up properly, the separation obtained should allow identification of the small amount the maturely present 18:1 11 cis isomers next to the 18:1 9cis peak in (high – temperature) refined oils such as soybean oil. The two 18:1c isomers should be clearly separated.

(b) The 20:1 natural isomer should be positioned exactly between the test eluting 18:3 trans isomers (trans, cis, cis) and the 18:3 cc (lionlenic acid) Peak in (high – temperature) refined oil.

(c) If the separation is sufficient for this type of analysis, in (high – temperature) refined oils a small peak for the 18:1 trans isomers, two approximately equally sized 18:2 trans isomers .and 4 sometime 5) 18:3 trans isomers should be obtained.

(d) For partially hydrogenated oil and fat, the separation of 18:1 13 trans and the 18:2 9 cis isomers should be visible on chromatogram. This required for an accurate peak split between cis and trans.

Peak Identification:

(a) For (high – temperature) refined oils and fats the trans isomers are limited in number because only geometric isomers, with DBCS on the same natural position, are formed. For C18 fatty acids these specific isomers are 18:1 9 t, 18:2 9c 12t and 9t 12c, and 18:3 tct, cct, ctc tcc9 12, 15 – isomers c in some samples the 18:2 9t 12t and 18:3 ttc isomers are found as well in very small amounts.

(b) For partially hydrogenated oils and fats the trans DB – containing isomers are identified use equivalent chain length (ECL). For accurate peak identification with this system the ECL values have to be determined after suitable calibration with available cis and trans fatty isomers standards.

Calculation and expression of results:

(a) Correct the area of each peak to compensate for the flame ionization detector (FID) response for each component. The FID correction factors are calculated from the molecular weight of the FAME as follows:

$$\mathbf{FID\ x} = \frac{\mathbf{MW\ X}}{(n_x - 1)\ (AW_C)\ (FID_{16:0})}$$

Where-

FID X = the FID factor for component x

MW x =molecular weight of component x

n x = the number of carbon atoms in the FAME of component x

Aw c = the atomic weight of carbon (12.01)

FID 16:0 =the FID correction factor for 16:0 (1.407)

All other FID correction factors used in the calculation are relative to FID. for example, the correction factor for 10:0 becomes 16:0 1.10.

3.5.13 Determination of saponification value:

The saponification value is the number of milligrams of potassium hydroxide required to saponify 1 g of fat under the conditions specified. It is a measure of the average molecular weight of all the fatty acids present.

Principle:

The glycerides present are split by alcoholic alkali and any free fatty acids are neutralized. Excess alkali is back – titration with hydrochloric acid in the presence of an indicator.

Scope:

Applicable to all normal oils and fats.

Reagents:

1. Potassium hydroxide, approximately 0.5 N solution in 95% (v/v) ethanol.
2. The solution should be colourless or straw yellow.

3. Phenolphthalein solution 1% (v/v) in 95% ethanol.
4. Boiling aids e.g. pumice stone.

Apparatus:

1. Conical flask, 250 ml capacity, made of alkali resistant glass, with ground neck.
2. Reflex condenser, with ground glass joint to fit the flask.
3. Water bath, or hot plate with variable heat control.
4. Burette, 50 ml capacity graduated in 0.1 ml divisions.
5. Pipette 25 ml.

Preparation of sample:

Melt and homogenize the sample if it's not liquid at 60 – 70º C before taking a test portion. Filter through a dry filter paper to remove any impurities.

Procedure:

1. Weight to the nearest 0.005 g, a test portion in to a conical flask such that the volume of hydrochloric acid required for the determination will about half that for the blank determination. This weight will usually be around 2 g.
2. Add 25 ml of ethanolic potassium hydroxide solution and some boiling aids.
3. Connect the reflux condenser and boil gently at least 60 minutes, swirling, the content of the flask from time to time.
4. Allow the flask and condenser to cool slightly before washing the inside of the condenser with a little distilled water. Add 1 ml of phenolphthalein solution and titrate with 0.5 N hydrochloric acid until the pink colour of the indicator just disappears.
5. Conduct a blank determination simultaneously with the test sample using the same procedure.

Expression of results:

The saponifaible value is given by

$$SV = \frac{56.1\ N\ (V_b - V_s)}{W}$$

Where:

V b : is the volume, in milliliter of the hydrochloric acid used for the blank.
V s : is the volume, in milliliter of the hydrochloric acid used for determination of the sample.
N: is the normality of the hydrochloric acid.
W: is the weight, in grams of the test portion.
Express the result to the nearest unit

Repeatability:

The difference between the result of two determination on the same test sample, carried out simultaneously or in rapid succession by the same analyst, should not exceed 0.5 % of their main value.

3.5.14 Saponification Followed by BF3-Catalyzed Methylation:

A lipid sample in a 10 mL glass balloon (48.6 mg) was hydrolyzed with 3 mL of NaOH methanolic solution (0.5%) at 60 °C for 20 min. The reaction mixture was methylated with 5 mL of BF3/MeOH (14%) and heated until ebullition for 5 min. Twenty milliliter of saturated NaCl aqueous solution was added to the reaction product and extracted with 20 mL of n-hexane. The organic phase was dried with 1 g of magnesium sulfate (Merck, Darmstadt, Germany) and filtered using a 0.45 µm nylon syringe filter. The excess of n-hexane was evaporated until 0.5 mL under a gentle stream of purified nitrogen (>99.5%).

3.5.15 GC-MS Analysis:

GC-MS analyses were carried out on an Agilent 6890 Series gas chromatograph equipped with a 5973 N mass selective detector (USA). A programmed temperature vaporization injector operating in the split mode (1:100) at 250 °C and a ZB-5 capillary column (30 m × 0.25 mm i.d. 0.25 µm film thickness; 5% diphenyl, 95% dimethylpolysiloxane; Phenomenex, USA) were used. The increase in the oven temperature was programmed from 80 °C (held for 1 min) to 300 °C (held for 10 min), at a rate of 20 °C/min. Helium was used as carrier gas at constant pressure (9.8 psi) and the injection volume was 1 µL. The transfer line, ion source and quadrupole temperatures were maintained at 280, 230 and 150 °C, respectively, and a solvent delay of 4 min was selected. Electron ionization was performed at 70 eV and the mass spectrometer was operated in the full scan mode in the range 35–550 Da. All results were compared with Wiley's library reference spectral bank (G1035B; RevD.02.00). Data recording and instrument control were performed by the MSD ChemStation software (G1701 CA; ver.C.00.00; Agilent Technologies, Little Falls, DE, USA.

3.5.16 Statistical analysis:

The results were expressed as mean ± SD. One-way ANOVA was used to analyze the variation and level of statistical significance between groups. $P < 0.05$ was considered statistically significant.

4. RESULTS

Analysis of methylated derivatives x identification of fatty acids:

The hexane fraction (FHEHXA) was subjected to saponification reaction (KOH / MeOH) to obtain the fatty acid salts (Saponification). These, after acidification, gave the free fatty acids which were then esterified, (MeOH/HCl) to yield the corresponding methyl esters (AGME). Silica gel column chromatography of the crude reaction product gave the fractions AGME 24-28, AGME 29-30 and AGME 31-39 (Methylation).

Analysis of AGME 24-28 in CG/MS showed the presence of ten fatty acids (Table 1), identified by the molecular ions [M] + (consistent with the respective molecular formulas) corresponding to the respective methyl esters represented by the peaks with retention (RT) and percentages (%) in the total ion chromatogram (Figure 1).

Table.1: Analysis of AGME 24-28 in CG/MS.

Substance	Common Name	T_R (min)	Content (%)	M.M	F.M
Haxadecanoic acid	Palmitic acid	23,16	1,46	256	$C_{16}H_{32}O_2$
Cis-octadec-9-enoic acid	Oleic acid	26,63	55,05	282	$C_{18}H_{34}O_2$
Octadeca-9-yn-11-trans-enoic acid	ximeninic acid	28,85	17,38	278	$C_{18}H_{30}O_2$
Cis-tetracos-15-enoic acid	Nervonic acid	36,64	3,00	366	$C_{24}H_{46}O_2$
Tetracosanoic acid	Lignoceric acid	36,99	2,05	368	$C_{24}H_{48}O_2$
Cis-hexacos-17-enoic acid	Ximenic acid	39,54	3,06	394	$C_{26}H_{50}O_2$
Hexacosanoic acid	Ceric acid	39,84	3,34	396	$C_{26}H_{52}O_2$
Cis-octacos-19-enoic acid	Acid Ximenixico[a]	41,81	9,85	422	$C_{28}H_{54}O_2$
Octacosanoic acid	Montanic acid	42,01	2,28	424	$C_{28}H_{52}O_2$
Trans-triacont-21-enoic acid	Lumequeic acid	44,13	2,22	450	$C_{30}H_{58}O_2$

Table 1: Fatty acids identified as methyl esters (AGME 24-28).
T_R: Retention time; M.M. Molar mass (g mol-1); F.M: Molecular formula.
a: common name used in this work.

Mass spectra (MS) of the methylated components were also compared to mass spectra of fatty acid methyl esters reported in the literature and were consistent with the proposed structures, exhibiting characteristic m/z ratio fragments, as described below. The main components were Z-octadec-9-enoic acid (55.05%), octadeca-9-in-11-trans-enoic acid (17.38%) and Z-docos-13-enoic acids known as oleic acids, ximeninic and cis-19-octacosenoic acids, respectively (Table 1).

The four saturated fatty acids (1, 5, 7 and 9) exhibited practically the same fragmentation pattern, with peaks due to breaks in the alkane chain [M-15 (CH_3) to M-183 ($C_{13}H_{27}$, 1), M -295 ($C_{21}H_{43}$, 5), M-323 ($C_{23}H_{47}$, 7) and M-351 ($C_{25}H_{51}$, 9), major peaks in *m/z* 41, 43, 55, 57, 74, 87 and 143 and base peak in m/z 74, the latter, resulting from Diels-Alder retro fragmentation.

1. T_R 23,164: M^+ *m/z* 270 (fórmula molecular $C_{17}H_{34}O_2$), 239 [$(M-31)^+$, perda de OCH_3], 227 $(M-43)^+$, 213 $(M-57)^+$, 199 $(M-71)^+$, 185 $(M-85)^+$, 171 $(M-99)^+$, 143 $(C_6H_{13}CO_2CH_3)^+$, 101 $(CH_2CH_2CH_2CO_2CH_3)^+$, 87 $(CH_2CH_2CO_2CH_3)^+$, 74 $[H_2C=C(OH)OCH_3]^{+\bullet}$, 55 $(H_2CCH=C=O)^+$, 57 $(C_4H_9)^+$, 43 $(C_3H_7)^+$ e 41 $(C_3H_5)^+$: Palmitic acid.

5. T_R 36,990: M^+ 382 (fórmula molecular $C_{25}H_{50}O_2$), 367 (M-15), 351 (M-31), 353 (M-29), 339 (M-43), 325 (M-57), 311 (M-71), 297 (M-85), 283 (M-99). Os picos principais foram em *m/z* 41 $(C_3H_5)^+$, 43 $(C_3H_7)^+$, 55 $(H_2CCH=C=O)^+$, 57 $(C_4H_9)^+$, 74 $(H_2C=C(OH)OCH_3)^{+\bullet}$, 87 $(CH_2CH_2CO_2CH_3)^+$ e 143 $(C_6H_{12}CO_2CH_3)^+$: Lignoceric Acid

.7. T_R 39,538: M^+ 410 (fórmula molecular $C_{27}H_{54}O_2$), 395 (M-15), 381 (M-29), 367 (M-43), 353 (M-57), 339 (M-71), 325 (M-85) e 311 (M-99). Os picos principais foram em *m/z* 41 $(C_3H_5)^+$, 43 $(C_3H_7)^+$, 55 ($(H_2CCH=C=O)^+$, 57 $(C_4H_9)^+$, 74 $(H_2C=C(OH)OCH_3)^{+\bullet}$, 87 $(CH_2CH_2CO_2CH_3)^+$ e 143 $(C_6H_{12}CO_2CH_3)^+$: Cerylic acid.

9. T_R 42,006: M^+ 438 (fórmula molecular $C_{29}H_{58}O_2$), 409 (M-29), 395 (M-43), 381 (M-57), 367 (M-71), 353 (M-85) e 339 (M-99). Os picos principais em *m/z* 41 $(C_3H_5)^+$, 43 $(C_3H_7)^+$, 57 $(C_4H_7)^+$, 55 $(H_2CCH=C=O)^+$, 74 $(H_2C=C(OH)OCH_3)^{+\bullet}$, 87 $(CH_2CH_2CO_2CH_3)^+$ e 143 $(C_6H_{12}CO_2CH_3)^+$; Mushroom Acid.

The five monounsaturated acids (2, 4, 6, 8 and 10) exhibited the same pattern of fragmentation, with the principal peaks in *m/z* 41, 43, 55, 69, 74, 83, 97, 98 and 111. It is worth highlighting in all these cases the peak due to the fragment in M-32 (M-CH3OH), as well as the base peak in *m/z* 55.

2. T_R 26,634, M^+ 296 (fórmula molecular $C_{19}H_{36}O_2$), *m/z*: 265 (M-31), 264 (M-32), 222 (M-74), 111 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2CH_2)^+$, 98 $(CH_3CH_2CH{=}CHCH_2CH_2CH_3)^+$, 97 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2)^+$, 87 $(CH_2CH_2CO_2CH_3)^+$, 83 $(CH_2CH_2CH_2CH_2CH{=}CH_2)^+$, 74 $([H_2C{=}C(OH)OCH_3]^+$, 69 $(CH_2CH_2CH_2CH{=}CH_2)^+$, 55 $[(CH_2CH_2CH{=}CH_2)^+$ e/ou $H_2C{=}CH\text{-}CO)^+]$, 43 $(C_3H_7)^+$ e 41 $(CH_2CH{=}CH_2)^+$; Oleic acid.
4. T_R 36.644, M 380 (fórmula molecular $C_{25}H_{48}O_2$), *m/z*: 349 (M-31), 348 (M-32), 306 (M-74), 111 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2CH_2)^+$, 97 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2)^+$, 83 $(CH_2CH_2CH_2CH_2CH{=}CH_2)^+$, 74 $([H_2C{=}C(OH)OCH_3]^+$, 69 $(CH_2CH_2CH_2CH{=}CH_2)^+$, 57 $(C_4H_9)^+$, 55 $[(CH_2CH_2CH{=}CH_2)^+$ e/ou $H_2C{=}CH\text{-}CO)^+]$, 43 $(C_3H_7)^+$, 41 $(C_3H_5)^+$; Nervous Acid.
6. T_R 39,538, M^+ 408 (fórmula molecular $C_{27}H_{52}O_2$), *m/z*: 377 (M-31), 376 (M-32), 334 (M-74), 111 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2CH_2)^+$, 97 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2)^+$, 83 $(CH_2CH_2CH_2CH_2CH{=}CH_2)^+$, 74 $([H_2C{=}C(OH)OCH_3]^+$, 69 $(CH_2CH_2CH_2CH{=}CH_2)^+$, 57 $(C_4H_9)^+$, 55 $[(CH_2CH_2CH{=}CH_2)^+$ e/ou $H_2C{=}CH\text{-}CO)^+]$, 43 $(C_3H_7)^+$, 41 $(C_3H_5)^+$; Ximenic Acid.
8. T_R 41,809, M^+ 436 (fórmula molecular $C_{29}H_{56}O_2$), *m/z*: 405 (M-31), 404 (M-32), 362 (M-74), 111 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2CH_2)^+$, 97 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2)^+$, 83 $(CH_2CH_2CH_2CH_2CH{=}CH_2)^+$, 74 $([H_2C{=}C(OH)OCH_3]^+$, 69 $(CH_2CH_2CH_2CH{=}CH_2)^+$, 57 $(C_4H_9)^+$, 55 $[(CH_2CH_2CH{=}CH_2)^+$ e/ou $H_2C{=}CH\text{-}CO)^+]$, 43 $(C_3H_7)^+$, 41 $(C_3H_5)^+$; Ximenoic acid (cis-19-octacosenoic acid).
10. T_R 44,132, M^+ 464 (fórmula molecular $C_{31}H_{60}O_2$), *m/z*: 433 (M-31), 432 (M-32), 390 (M-74), 111 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2CH_2)^+$, 97 $(CH_3CH_2CH{=}CHCH_2CH_2CH_2)^+$, 83 $(CH_2CH_2CH_2CH_2CH{=}CH_2)^+$, 74 $([H_2C{=}C(OH)OCH_3]^+$, 69 $(CH_2CH_2CH_2CH{=}CH_2)^+$, 57 $(C_4H_9)^+$, 55 $[(CH_2CH_2CH{=}CH_2)^+$ e/ou $H_2C{=}CH\text{-}CO)^+]$, 43 $(C_3H_7)^+$, 41 $(C_3H_5)^+$;Lumequeic Acid.

In the case of the component with retention time 28,853 (3) a distinct fragmentation pattern was observed. Except for the peaks in *m/z* 41 and 43, fragments common to all and the peak in *m/z* 55, observed in the unsaturated acid spectra 2, 4, 6, 8 and 10, the mass spectrum of 3 showed the peaks *m/z* 67, 79, 80, 93 and 150, with the base peak in *m/z* 79. The highlights were the peaks due to fragments in *m/z* 261 (M-31 loss of OCH_3), 219 -CH_2CH_2CH = CH (CH_2) 5 $CH_3]$ + en / z 150 $[H_2$ C = CHCH = CH (CH_2) 5 $CH_3]$ +, both indicative of a C_{18}-enine structure with the triple bond between C_9 and C_{10} carbons [10,11]. The base peak in *m/z* 79 (C_6H_7) + indicated the presence of more than one double or triple bond in the fatty acid chain [12].
3. 28,853, M^+ 292 (fórmula molecular $C_{19}H_{32}O_2$), *m/z*: 261 (M-31), 219 (M-73), 164, 150, 135 $([H_2C{=}C{=}CHCH{=}CH(CH_2)_5]^+$, 121 $[[H_2C{=}C{=}CHCH{=}CH(CH_2)_4]^+$, 107 $[H_2C{=}C{=}CHCH{=}CH(CH_2)_3]^+$, 93 $[H_2C{=}C{=}CHCH{=}CH(CH_2)_2]^+$, 79 $[H_2C{=}C{=}CHCH{=}CHCH_2]^+$, 67 $[HCC(CH_2)_3]^+$, 55 $(C_3H_3O/C_4H_7)^+$, 43 $(C_3H_7)^+$ e 41 $(C_3H_5)^+$; Ximeminic Acid.

The structures of the fatty acids identified in the seeds of *X. americana* are shown in Figure 2. The fractions AGME 29-30 and AGME 32-39 (Methylation), By GC/MS analysis, practically led to the same constituents of the AGME fraction 24-28.

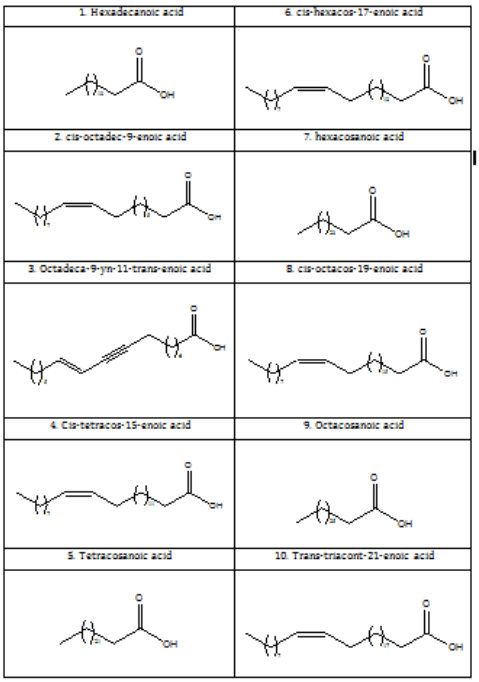

Figure 2: Structures of fatty acids identified in Seeds of *X. americana*.

Plants are the major source for discovering new compounds with medicinal value for drug development [66-69]. In chromatography methods, gas chromatography (GC) is one of the most widely used techniques and has become one of the most important tools for the separation of phytocompounds. In the last few years GC-MS has become firmly established itself as a powerful technique for identification of secondary metabolites in both plant and non-plant species [70-73]. In the present study solvent extracts of *X. americana*., ethanol extracts was subjected to GC-MS analysis. GC-MS spectrum confirms the presence of various bioactive compounds in extract of *X. americana* with different retention time. The gas chromatogram shows presence of relative concentration of various compounds present in *X. americana* getting eluted at different retention time [74-77]. The peak height represents relative concentration of components present in *Ximenia americana*. The mass spectrometer helps in the identification of compounds eluted at different retention time. The major phytoconstituents were found to be *Oleic acid*, *n-Hexadecanoic acid*, *Non-decanoic acid and Octadecatrienoic acid* in chloroform extract; and *3-Undecene*, *Tridecene*, *Trifluoroaceteoxy tetradecene*, *Trans-11Tetradecenylacetate and Trichloroacetic acid-3-tridecyl-ester* in ethyl acetate extract; where in *1-Tetracosanol*, *Behenyl alcohol*, *1-Hexacosanol*, *Octadecanal*, *4-Piperidine propanoic acid and α –D-mannofuranoside* in methanol extract; *8,11,14-Eicosatrienoic acid*, *7-Tetradecanal and 1-Ocytyn-3-Ol-4-Ethy in ethanol extract*; aqueous extract showed the presence of *9,12-Octadecandionoic acid*. The above mentioned identified compounds are known to possess several biological activities and industrial applications such as n-Hexadecanoic acid was known to have antioxidant, anti-inflammatory, hypocholesterolemic and cancer prevention activities [78]; tetradecanoic acid was known to have antibacterial and antifungal activity [79]; *9,12-Octadecandionoic acid was* reported to have anti-inflammatory, antibacterial, hypocholesterolemic and hepatoprotective activity [80]; oleic acid was proven to having antibacterial activity and industrial application as emulsifying agent [81]. Overall these phytoconstituents have been shown to possess different biological activities and industrial applications such as antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, dietary nutrient, in perfume making and alcohol industry [81-85]. However no reports are available on the activities of some identified compounds such as 3-undecene, tridecene, and octadecanal.

Table 2. X. americana seeds oil properties.

Colour	yellow
Odour	Odourless
Taste	Tasteless
liquid State	Liquid
Solubility	Freely soluble in non polar solvents
Boiling point	157 C
Density	0.9376 g/ml
Viscosity 25 C	227.58
Viscosity 70 C	42.0
Refractive index	1.4770
Acid value	0.2805
Ester value	9.82
Iodine value	47.59
Peroxide value	30
Saponification value	11.43
Ratio value	35.009

The results of GC-MS profile can be used as pharmacognostical tool for the identification of novel drugs from *Ximenia americana*. This study provides scientific evidence that oil of *Ximenia Americana* seed has antioxidant efficacy. Thus, considering its relative hypoglycemic potency, they may serve as useful therapeutic agents trail for treating diabetes and other chronic diseases. The present studies also supporting and provide a clear scientific basis that *X. americana* can be source for the novel medicines.

Further, purification of the specific active constituents needs to be carried out, that can be used for the discovery of novel drugs to treat many diseases a worldwide epidemic disease and can also be used as antibacterial and anticancer.

5. DISCUSSION

From an extensive literature review was observed that the Ximenia americana is widely used as a popular alternative remedy in certain regions of some countries of the Africa (Guinea, Ethiopia, Nigeria, Sudan) and in the Brazil. The plant, used by their crude extracts, especially, aqueous and methanolic, showed several biological activities such as antimicrobial, antifungal, anticancer, antitrypanosomal, antirheumatic, antioxidant, analgesic, moluscicide, pesticidal, antipyretic, antifugal, among others. There are several papers in the literature confirming these activities. The crude extracts consist of complex mixture of compounds called secondary metabolites produced by plants, which include, mainly, flavonoids, saponins, alkaloids, quinones, terpenoids, phenols, glycosides and sterols. Many plants have a

prolonged and uneventful use that may serve as indirect evidence to their efficacy. However, in the absence of objective proof of efficacy and without the knowledge of the constituents responsible for the physiological actions, the validity of the remedies is questionable and its use restricted. It generally was observed that the more the constituents in a given species, the more diverse the micro-organisms it acts upon. The difference of activity appears to be directly related to the qualitative and/or quantitative diversity of the compounds that are being accumulated by the plants investigated.

In the present study, the phytochemical analysis helped in the detection of the secondary metabolites in the selected extracts of the plant. Phytoconstituents were evaluated by different quantitative biochemical tests. Natural antioxidants play an important role in the enhancement of antioxidant capacity of the blood plasma and help in the prevention of many diseases including cancer and diabetes [86]. Especially phenols and polyphenols are the main secondary metabolites present in a plant which acts as antioxidant or free radical scavengers [87]. Phenols are also known to have several biological activities such as anti-inflammatory, anti-tumor and antioxidant activities [88, 89]. Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids [90]. The presence of phenols, flavonoids and tannins was observed in all solvent extracts except chloroform extract, whereas the presence of oils and fats was common in all solvent extracts. The study also reveals that the detected phytochemicals in seed of X. americana are pharmaceutically important. These secondary metabolites are considered as natural source of antioxidant, antimicrobial and anti-inflammatory agents which have been shown to reduce the risk and progression of many diseases such as cancer and diabetes [91, 92]. Especially phenolic compounds are high potent radical terminators by donating hydrogen atom to radical and inhibit lipid oxidation. Polyphenolic compounds present in plant are known to have several biological activities like antitumor, anti-inflammatory, antioxidant and anticancer [93, 94]. In other work, aqueous extract of leaves of X. americana was found to be rich in total phenolic content (91.04 mg/g GAE). Antioxidants are compounds that protect cells against the damaging effect of reactive oxygen species [95].

Recently natural antioxidants are in high demand because of their potenitial in health promotion and disease prevention. It is well known that antioxidant properties of the plant extracts cannot be evaluated by one single method due to the complex nature of phytochemicals and needs a further method.

In recent study by Widad [10] were found a total of 23 mostly phenolic compounds were detected in a methanol leaf extract of *X. a.* var. *caffra*. The leaf extract showed substantial antioxidant activity *in vitro* and *in vivo* using the *C. elegans* animal model. It also inhibited four crucial enzymes of skin aging (collagenase, elastase, hyaluronidase, and tyrosinase) and the biofilm formation of *P. aeruginosa*. To sum up, the leaf extract exhibits interesting dermato-cosmeceutical properties that might be attributed to its antioxidant and antibacterial activities. Current findings could provide scientific evidence for possible utilization of the *X. a.* var. *caffra* leaf extract and its compounds as an antiaging agent.

6.1 CONCLUSION:

Analysis of the oil of *X. americana* seeds by gas chromatography coupled to mass spectrometry using the saponification/methylation route allowed the identification of ten fatty acids. Four of these acids (palmitic, oleic, ximeninic and lignoceric) had already been identified by analyzing the oil of these seeds via derivatization by silylation, also by gas chromatography coupled to mass spectrometry. It was possible to identify other six fatty acids (ceric, nerve, montane, ximenic, oxymic and lumeochemical), as well as to confirm the presence of the first four, contributing significantly to the knowledge of the composition of the oil of the species under study. It is worth mentioning the high content (19.77%) of total proteins and the high percentage (about 27.71%) of oil in the seeds of the edible fruits of this plant species, which, together with its spread in Northeast Brazil, possible use as a nutraceutical, in cosmetology or even for the production of alkyl fatty acid monoesters (biodiesel). In addition, the present study contributed to increase knowledge about the medicinal potential of *X. americana*. Cataplasms of plants of the genus Ximênia are used as masks for treatment of the skin. The ximeninic acid and its ethyl ester (ximenoil) have topical action, being used in the form of emulsions with anti-cellulite action.

6.2 Recommendations:

Further study requires purification, characterization and structural elucidation of phenolic compounds in oil extracts that may help in the development of new phytopharmaceuticals.

Needs more researches in Ximenia Americana seed and other parts of fruits as anti-inflammatory, anti cancer and uses in drugs industry as antibiotics in addition to great role in cosmetics used.

Declarations

Authors' contributions: All authors contributed equally

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