Sudan University of Sciences and Technology

College of Graduate Studies

Chemical and Biological Study of Some Compounds Extracted from *Cajanus cajan* , *Zea mays* and *Guera senegalensis*

استهلال

(أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَسَلَكَهُ يَنَابِيعَ فِي الْأَرْضِ ثُمَّ يُخْرِجُ بِهِ زَرْعًا مُخْتَلِفًا أَلْوَانُهُ ثُمَّ يَهِيجُ فَتَرَاهُ مُصْفَرًّا ثُمَّ يَجْعَلُهُ حُطَامًا ۚ إِنَّ فِي ذَٰلِكَ لَذِكْرَىٰ لِأُولِي الْأَلْبَابِ)(الزمر:٢١)

Dedication

To

My parents

Brothers and Sisters

Acknowledgement

I am grateful to Almighty Allah for the will and blessings in completing this work.I would like to express my thanks to Professor Mohamed Abdel Karim Mohamed ,my supervisor, who gave me the chance to do this wonderful research. He also helped me in being engaged with up-to-date literature in the field of research.I also would like to thank my parents and friends who helped me a lot in finalizing this research within the limited timeframe.

My special thanks are extended to the staff of Chemistry Department,Sudan University of Science and Technology, for their support and encouragement throughout my study.

Abstract

In this study *Cajanus cajan*(L.),*Guera senagalensis* and *Zea mays* were investigated.Different fractions from *Cajanus cajan*(L.) and *Guera senagalensis* were evaluated for their biological activity.The antimicrobial potential was measured via the cup plate agar diffusion assay and significant results were obtained .The ethanolic extracts of the targeted species were subjected to phytochemical screening which revealed the presence of some important phytoconstituents.

A Fixed oil was extracted from *Zea mays* seeds via solvent extraction.The oil was then analyzed by GC-MS where 20 constituents were detected. This oil was also evaluated for its antimicrobial activity and moderate activity was observed.

خلاصة البحث

في هذه الدراسة اخضعت اوراق نبات العدسية ، اوراق الغبيش ونبات الذره الشامى للمسح الفيتوكيميائى حيث اتضح وجود بعض المكونات الهامه بايولوجيا.

ثم اختبرت فعاليه مستخلصات هذه النباتات ضد بعض انواع البكتريا وكانت الفعاليه لهذه المستخلصات قويه. ايضا استخلص زيت الذره الشامى واخضع لاختبارات بيولوجيه حيث اعطى فعاليه ضد بعض البكتريا. ثم حددت مكونات الزيت بتقنيه الكروموتوغرافيا السائله- طيف الكتله حيث اتضح وجود عشرون مكونا.

1-Introduction

1.1- *Zea mays*

Zea mays subsp. *Mays* from Spanish: *maíz* after Taíno *mahiz*), known in some English-speaking countries as **corn**, and in the United Kingdom as **sweet corn**, is a large grain plant domesticated by indigenous peoples in Mexico in prehistoric times about 10,000 years ago[1].

The leafy stalk produces separate pollen and ovuliferous inflorescences or ears which are fruits yielding kernels (often erroneously called seeds). Maize kernels are often used in cooking as a starch. The six major types of maize are dent, flint, pod, popcorn,flour, and sweet[1].

The maize plant is often 3 m (10 ft) in height,[19] though some natural strains can grow 12 m (39 ft).[20] The stem is commonly composed of 20 internodes [21] of 18 cm (7.1 in) length. A leaf grows from each node, which is generally 9 cm (4 in) in width and 120 cm (4 ft) in length[1].

Ears develop above a few of the leaves in the midsection of the plant, between the stem and leaf sheath, elongating by ~3 mm/day, to a length of 18 cm (7 in) [23] with 60 cm (24 in) being the maximum alleged in the subspecies.[24] They are

femaleinflorescences, tightly enveloped by several layers of ear leaves commonly called husks. Certain varieties of maize have been bred to produce many additional developed ears. These are the source of the "baby corn" used as a vegetable in Asian cuisine[1].

The apex of the stem ends in the tassel, an inflorescence of male flowers. When the tassel is mature and conditions are suitably warm and dry, anthers on the tassel dehisce and release pollen. Maize pollen is anemophilous (dispersed by wind), and because of its large settling velocity, most pollen falls within a few meters of the tassel.Elongated stigmas, called silks, emerge from the whorl of husk leaves at the end of the ear.

Due to the effectiveness in treating various ailments, Zea mays hair is frequently chosen and worldwidely used as an old folk therapeutic agent. Zea mays belongs to family Gramineae. It can be found in tropical regions for instance North American, China, India and various parts of the world including Malaysia. Though, Zea mays crop production in Malaysia is not significant. Zea mays hair is found inside the husks of corn. It hardly shows themselves until the emergence of the pale yellow silks from the end of the husks. The silk are elongated stigmas that resemble bunch of hair[2].

Zea mays hair contains various bioactive constituents comprise of protein, vitamin, minerals and salts , flavonoids, steroid , carbohydrate and volatile components[3]. Phytochemicals present

showed potential activities against hypoglycaemic[4]. On the other aspect, Zea mays hair extract has been reported to increase insulin level and healed injured β-cell. *Zea mays* hair also has been claimed to have immunology activity. It is said to treat hypersensitivity related to type I allergy disease[5,6]. Besides that, *Zea mays* hair has been documented to exhibit anti-proliferative effect on cancer cell line[7].

Zea mays hair has been claimed to have effect more particularly on renal diseases including chronic nephritis, benign prostate hyperplasia, gout and cystitis[8] .It helps to pass stone from kidney and urinary tract and prevent the inflammatory effect. Besides, *Zea mays* hair has anti-prostatitis and anti-spasmodic activities[9] . Recently, *Zea mays* hair has been reported to have anti-fatigue activity. The flavonoids compound in the hair has affected the mechanism in the blood. Hence it increased the hepatic glycogen and consequently, increased the exercise tolerance[10] .The hair has antioxidative properties. They protect cells from damages due to oxidation process in the body triggered by free radicals[11].

Zea mays

1.2-The genus *Cajanus*

The genus ***Cajanus*** is a member of the plant family Fabaceae. There are 37 species, mainly distributed across Africa, Asia and Australasia.Species include the pigeon pea (*Cajanus cajan*).

Cajanus species are used as food plants by the larvae of some *Lepidoptera* species including *Endoclita malabaricus*[46].

Cajanus cajan L. is an important legume used as nutrient and rich source of vitamins,proteins and minerals. It is used as traditional medicine since ancient times. *Cajanus cajan* is used for the treatment of hepatitis,dysentery,diabetes and measles. Its leaves are used to treat wounds,malaria,bedsores and alcohol-induced liver damage.It is antioxidant and antibacterial[12].

Phytochemical analysis showed the presence of saponins,tannins,alkaloids and flavonoids.

Cajanus cajan

Biosystematic studies encompassing morphocytological and electrophoretic analyses of *Cajanus cajan*, seven species of *Atylosia* and one of *Rhynchosia* revealed that *A. cajanifolia* is closest to *C. cajan*, followed by *A. lineata, A. scarabaeoides, A. sericea, A. albicans, A. volubilis, A. platycarpa* and *R. rothii*, in that order. A revision has been suggested for the taxonomic placement of the seven *Atylosia* species. Regarding the evolution of cultivated *C. cajan*, three possible alternatives have been suggested. Firstly, *C. cajan* could have evolved through gene mutation in *A. cajanifolia*; secondly, some of the *Atylosia* species and pigeonpea probably evolved from the same source;

and thirdly, the pigeonpea might have developed from naturally occurring interspecific crosses of *A. lineata* and *A. scarabaeoides*[12].

Cajanus scarabaeoides is a flowering plant in the genus *Cajanus* .Of the 32 different species within the genus *Cajanus*, only one, *C. cajan* (pigeonpea), is cultivated. *Cajanus scarabaeoides* is the closest wild relative to *C. cajan*, and is one of the easiest wild species to cross with pigeonpea cultivars.[2] *C. scarabaeoides* is found naturally in both temperate and tropical zones around the globe[12].This species has higher levels of drought tolerance, is found to have greater protein content, and has higher levels of resistance to insect pests compared to cultivated types[13] .

In China, *C. scarabaeoides* is sometimes used as fodder, and has shown to be effective in reducing diarrhea in cattle. In addition, the leaves of the plant species have been used to improve indigestion in traditional medicines as well as limit the excessive production of urine[14] .

1.3-The genus *Guiera*

Guiera senegalensis **J. F. Gmel**. (Combretaceae) is one of the most important West African medicinal plants, often used to treat a variety of microbial infections. The most frequently used plant part is the leaf, its medicinal use being corroborated by several in vitro antimicrobial activity studies. In Sudan leaves of

Guiera senegalensis are used in folkmedicine to treat many diseases including diabetes mellitus and certain infectious diseases. Often used in the form of popular products prepared in ways such as water soaking or cooking (infusion or decoction)[15].

Guiera senegalensis J.F.Gmel. (Combretaceae) is a shrub of the savannah region of West and Central Africa . Its leaves , 3-5 cm long and 1.5 – 3 cm broad , are opposite or suboppoite , oblong – epitic , rounded or slightly cordate at base , mucronate at apex. They are softly tomentose on both surfaces , with scattered black beneath[15].

Guiera senegalensis is widely used in the traditional medicine of western and central Africa[16] . In Sudan it is used as a remedy for malaria . In Ghana the leaves are used against disentry , diarrhoea, gastrointenstinal pains and disorders, rheumatism and fever[17]. Previous investigation into the prostaglandin biosynthesis showed a small inhibitory activity[18]. Mucilagines , tannins , flavonoids , alkaloids and amino acids are ,so far, known constituents of *Guiera senegalensis*[19]. From the methanolic extract of the leaves of this plant , flavonol aglycones as well as flavonol glycosides , some of them acylated , were isolated[20].

Guiera senegalensis

1.4-Essential oils (EOs)

The greatest use of EOs in the European Union (EU) is in food (as flavourings), perfumes (fragrances and aftershaves) and pharmaceuticals (for their functional properties). The well-known use of EO in aromatherapy constitutes little more than 2% of the total market. Individual components of EOs are also used as flavourings, either extracted from plant material or synthetically manufactured[21].

The antibacterial properties of essential oils and their components are exploited in such diverse commercial products as dental root canal sealers, antiseptics and feed supplements for lactating sows and weaned piglets . A few food preservatives containing EOs are already commercially available. 'DMC Base Natural' is a food preservative produced by DOMCA S.A. (Alhendín, Granada, Spain) and comprises 50% essential oils from rosemary, sage and citrus and 50% glycerol[21]. 'Protecta One' and 'Protecta Two' are blended herb extracts produced by

Bavaria Corp. (Apopka, FL, USA) and are classed as generally recognized as safe (GRAS) food additives in the U.S.A. Although the precise contents are not made known by the manufacturer, the extracts probably contain one or more EOs and are dispersed in solutions of sodium citrate and sodium chloride, respectively. Further physiological effects of EOs are made use of in widely differing products such as commercial potato sprout suppressants and insect repellents[21].

Steam distillation is the most commonly used method for producing EOs on a commercial basis. Extraction by means of liquid carbon dioxide under low temperature and high pressure produces a more natural organoleptic profile but is much more expensive[21].The difference in organoleptic profile indicates a difference in the composition of oils obtained by solvent extraction as opposed to distillation and this may also influence antimicrobial properties. This would appear to be confirmed by the fact that herb EOs extracted by hexane have been shown to exhibit greater antimicrobial activity than the corresponding steam distilled Eos[21]. EOs are volatile and therefore need to be stored in airtight containers in the dark in order to prevent compositional changes.Numerous publications have presented data on the composition of the various EOs..Detailed compositional analysis is achieved by gas chromatography and mass spectrometry of the EO or its headspace[21]. EOs can comprise more than sixty individual components. Major

components can constitute up to 85 % of the EO whereas other components are present only as a trace. The phenolic components are chiefly responsible for the antibacterial properties of EOs . There is some evidence that minor components have a critical part to play in\ antibacterial activity, possibly by producing a synergistic effect between other components. This has been found to be the case for sage, certain species of *Thymus* and oregano[21].

1.5-GC-MS Analysis

For over half a centery ,GC has played fundamental role in detecting how many compounds and in what proportion they exist in a mixture .However,the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced and requires a spectroscopic method of detection system.The most used is the mass spectrometric detector(MSD) which allows obtaining the "fingerprint" of the molecule i.e.its mass spectrum. Mass spectra provide information on the molecular weight ,elemental composition if high resolution mass spectrocmeter is used,functional groups and spatial isomers of the molecule[22].

The sample to be analyzed by GC-MS may be a liquid solution or a collection of molecules adsorbed on a surface e.g. the solid phase microextraction system.During the transfer into the GC ,sample is volatilized by rapid exposure to a zone kept at relatively high temperature(200-300^{o}C) and mixed with a

stream of carrier gas(Ar,He,Ne of H).The resulting gaseous mixture enters the separation section , chromatographic column which in its current version is a fused –silica tubular capillary coated internally with a thin film.Upon their displacement through the column, the analyte molecules are partitioned between the gas carrier stream(mobile phase) and the polymer coating (stationary phase), to an extent which depends mainly on their chemical structure[22] . At the end of the separation section, the molecules reach a detection system in which a specific physical property(thermal conductivity) or a physicochemical process (ionization in a flame ,electron capture) gives rise to an electron signal which is proportional to the amount of molecules of the same identity. A data system permits the processing of these data to produce a graph of the variation of detector system with time(chromatogram). Four principal sections are distinguishable in the chromatograph: introduction (injector) ,separation(chromatographic column),detection and data handling unit.Each section has its own function and its responsibility for the quality of the analysis and the results obtained. The injectin system , for example ,should ideally transfer the sample to the column quantitatively without discrimination on molecular weight or volatility and without chemical alteration(decomposition or isomerization). It is a critical step , especially for quatitative analysis.For correct GC operation , among other conditions , this gateway to the

column should remain unpolluted,clean, inert and leak-free.The main requirement the analyte in GC is that it should be volatile enough to be present in detectable amount in the mobile phase. Substances with low vapour pressure will not enter the chromatographic column and will accumulate at the injection system[22].

One of the most important characteristics of the chromatographic column is its resolution, or its ability to separate components with very similar distribution constants between the mobile and stationery phases(K_D). Chromatographic resolution is a function of many operational parameters. Among them, the nature of the stationery phase,mobile phase , temperature, the size of column(length,inner diameter and the thickness of the stationery phase). As the number of components in the mixture increases and the structure similarity between its components grows(isomerism) longer column are required for complete separation of components.Alternatively,for the same purpose one can employ smaller internal diameter columns. Obviously, increasing the length of the column markedly increases the analysis time. So the analysis of polyaromatic hydrocarbons and controlled drugs is regularily accomplished by using a 30m long column[22].

Highly polar,thermlabile ,ionic and high molecular weight compounds are not compatible with regular GC analysis.Depending on the molecular structure of the analyte and the functional groups available ,it is possible in some cases to obtain chemical derivatives which has a high vapour pressure. Sample preparation for GC involves technologies which preferentially isolate volatile and semi –volatile substances and prevents the presence of ionic and high molecular weight species in the mixture to be injected in the GC.These procedures can be divided roughly into three major groups: distillation, extraction and headspace methods.The resulting extracts or distillates are volatile mixtures suitable for GC and GC-MS analysis , but the mixture may need drying(anhydrous sodium sulphate) prior to injection into the chromatograph[22]. However,these techniques ,in general , are not suitable for studying and isolating compounds at trace level. In order to improve extraction efficiency and substantially reducing distillation time ,microwave – assisted hydrodistillation[23,24] is a common example of a laboratory-scale technique for essential ils and other volatile mixtures isolation. Such microwave-asissted distillation requires quarter the time employed for conventional heating .

1.6-The flavonoids

Phytochemicals are substances found in plants. Some phytochemicals- like flavonoids- exhibit a potential for

modulating human metabolism in a manner beneficial for the prevention of chronic and degenerative diseases[25].

Plant phenolics are important secondary metabolites present in plants[26] and are also responsible for their antioxidant action and various beneficial effects in a multitude of human diseases[27,28].Polyphenols are classified into :

i- Phenolic compounds: Are aromatic organic compounds with at least one hydroxyl group attached directly to a benzene ring. These are hydroxylated derivatives of benzoic acid, present in form of esters and glycosides.

ii- Phenolic acids: cinnamic acid derivatives. Often present in esterified form.

iii-Glycosidic phenylpropanoid esters[29,30].

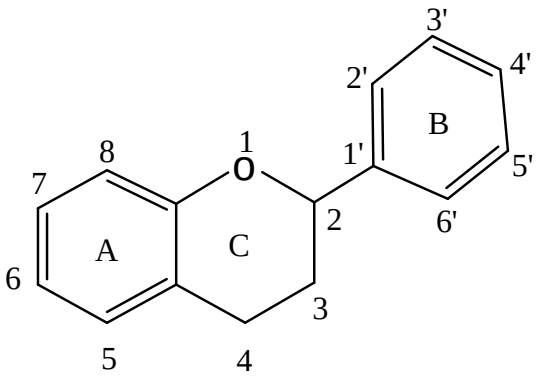
On the basis of C-skelton, polyphenols are classified as:

1. Flavonoids
2. Phenolic acids

Flavonoids are low molecular weight[31,32] bioactive polyphenols[33] which play a vital role in photosynthesising cells[34]. The original "flavonoid" research apparently began in 1936, when Hungarian scientist Albert Szent-Gyorgi was uncovering a synergy between pure vitamin C and as yet unidentified co-factors from the peels of lemons, which he first called "citrin," and, later wrongly, "vitamin P"[35].

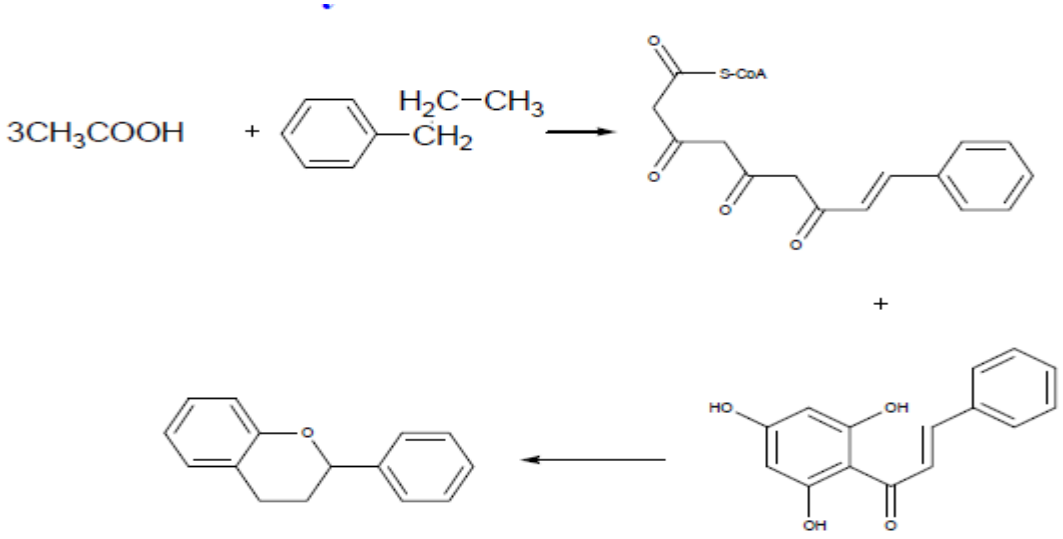
Flavonoids are secondary metabolites characterised by flavan nucleus and a C_6-C_3-C_6 carbon skeleton[36,37]. These are group of

structurally related compounds with a chromane-type skelton whichmay have a phenyl substituent in C_2- or C_3 position[38]. The basic structural feature of flavonoid is : 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C) as shown in basic structure of flavonoids (flavan nucleus) **(1)**.



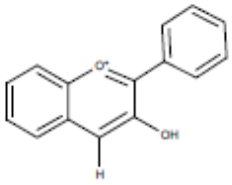
(1)

Biosynthesis of flavonoids (flavan nucleus) is shown in scheme 1.1.



Scheme 1.1: Biosynthesis of flavonoids (Flavan nucleus)

Flavonoids differ in their arrangement of hydroxyl, methoxy and glycosidic side groups and in the conjunction between A and B rings. A variation in C ring provides division of subclasses. According to their molecular structure, they are divided into eightclasses[39]:flavone**(2),**flavonones**(3)**,flavonols**(4)**,isoflavone**s(5)**,anthocyanidins**(6)**,catechins**(7),**dihydroflavonols(8) and chalcone**s(9)** .



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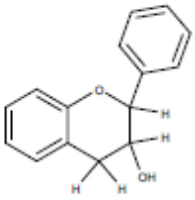

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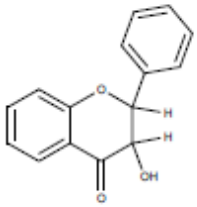

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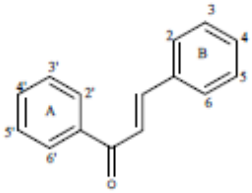

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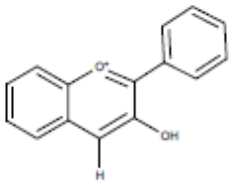

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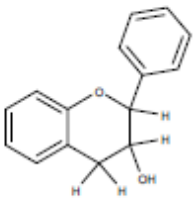

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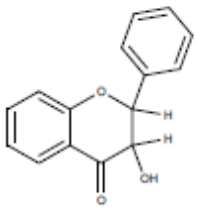

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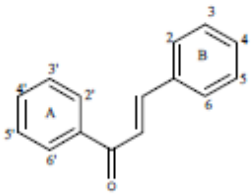
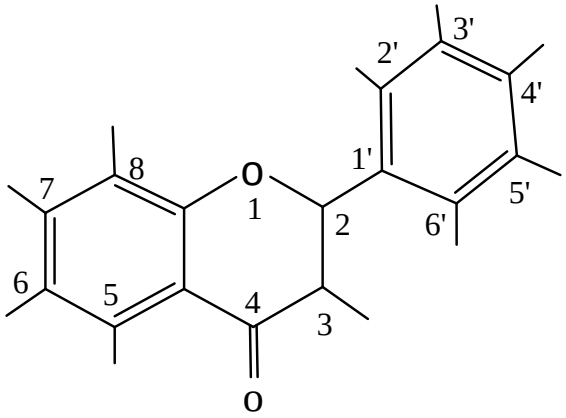


(9)

In plants, flavonoids are often present as O-glycosides or C-glycosides. The O-glycosides possess sugar substituent bound to –OH of aglycone, usually at position 3 or 7, whereas, C-glycosides possess sugar groups bound to carbon of aglycone usually 6-C or 8-C[39].



(10)

The actual number of flavonoids that have been found so far and for which the structure has been completely elucidated is large, but probably does not exceed 1% of the theoretical number of possible variants. This abundance of variants is further augmented by the chirality of the subunits and their connections. Since many stereoisomers do not differ significantly in their electronic or fluorescence spectra so the optical activity of the species is often a useful analytical parameter[40].

Flavonoids are widely distributed among the plant kingdom.Flavonoids are found in vegetables, fruits, nuts, seeds,

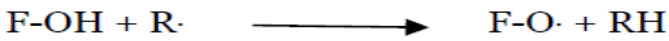
stem, flowers, tea… etc. These are an integral part of our daily diet[41-43]. The dietary intake of flavonoids is estimated to be 1-2 g/day. The average intake of flavonols and flavones was found to be 23 mg/day, among which, flavonol quercetin contributed 16 mg/day[44].

Anthocyanin pigment present in flowers provide colour to it contributing to pollination[45]. Flavonoids present in leaves promote physiological survival of plant by protecting it from fungal infections and UV radiations. In addition, flavonoids are involved in photosensitisation, energy transfer, respiration and photosynthesis control, morphogenesis, sex-determination and energy transfer.

Flavonoids have been reported to exert wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic, antitumour, treatment of neurodegenerative diseases, vasodilatory action. In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation. They are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase[46].

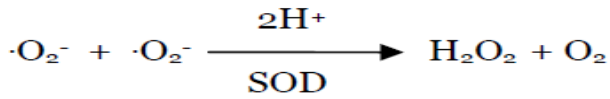
Flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers. This activity is attributed to their hydrogen-donating ability. Indeed, the phenolic groups of flavonoids serve as a source of a readily available ‘‘H” atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure.

Free radical scavenging capacity is primarily attributed to high reactivities of hydroxyl substituents that participate in the reaction as shown below:



Flavonoids inhibit lipid peroxidation *in vitro* at an early stage by acting as scavengers of superoxide anion and hydroxyl radicals. They terminate chain radical reaction by donating hydrogen atom to a peroxy radical as shown below; thus, forming flavonoids radical, which, further reacts with free radicals thus terminating propagating chain.

Naturally, the organism has developed a defence against toxic substances such as peroxynitrite and nitrous acid. An important mechanism is catalyzed by the enzyme superoxide dismutase (SOD), which converts two superoxide anions to H_2O_2 and O as shown below:



Aim of this study

This study was aimed to:

-Fractionation of ethanolic extracts of *Cajanus cajan* and *Guera senegalensis.*

-Evaluation of the biological activity of the different fractions.

-Extraction of Zea mays oil.

-GC-MS analysis of the oil

-Evaluation of oil for biological potential.