International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Review Article **CODEN: IJPNL6**

REVIEW ON *CYPERUS ESCULENTUS* : FROM FOOD SAFETY TO PHARMACOTHERAPEUTICS

Dhouha Krichène*[a], Diana Ansorena Artieda[b], Mokhtar Zarrouk[a] and Iciar Astiasarán[b]

[a] Olive Biotechnology Laboratory, Biotechnology Center of Borj Cedria, Technopole of Borj Cedria, BP.901-2050 Hammam-Lif, Tunisia.
[b] Department of Food Science and Nutrition, Physiology and Toxicology, Faculty of Pharmacy, University of Navarra, Irunlarrea sn, 31008, Pamplona, Spain.

***Corresponding author e-mail:** dhouha-krichene@hotmail.fr

Received on: 07-01-2016; Revised on: 08-02-2016; Accepted on: 25-02-2016

ABSTRACT

Plants are potent biochemical factories and have been components of phytomedicine. Plant based natural constituents can be derived from any part of the plant (leaves, flowers, roots, fruits, seeds, etc.) which contain active principles. It is well known that traditional herbal medicines existed before the application of the modern scientific methods to health care; and even today majority of the world population depends on herbal health care practices. *Cyperus esculentus L*. is distributed mainly in the Southern European region and in the Western part of Africa. Its sweet tubers are highly appreciated for their nutritive value with a high content of fiber, proteins, and sugars. They are rich in oleic acid, glucose, and vitamins C and E. It is often a utile plant largely utilized in traditional system of medicine and exhibits anti-inflammatory, anti-arthritic, analgesic, antidiarrhoeal, antimicrobial, antioxidant and various other activities. Our present review subsumes on chemical and biological properties of *Cyperus esculentus*. This compiled data reflects the therapeutic properties of this potential herb.

Keywords: *Cyperus esculentus*, Phytochemistry, Food applications, Multi potential bioactivities.

INTRODUCTION

Herbal medicine is a major component in all traditional medical systems, and is used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry, and represent a substantial proportion of the global drug market.

Tunisia is a rich source of medicinal plants, and a number of plant derived extracts are used to fight against various diseases. Only few plants have been scientifically explored. Plant derived natural products such as alkaloids, tannins, terpenes and flavonoids have received considerable attention in the recent years due to their diverse pharmacological properties including their analgesic, anti-inflammatory and antioxidant activities.

A perfect example of medicinal plant credited with innumerable medicinal qualities validated by modern science and used in folkloric medicine is *Cyperus esculentus Linn*. (Family: *Cyperaceae*, Common Name: yellow nutsedge and Tiger nut, Tunisian local name: Habb el aziz and El Saâd, Spanish local name: Chufas). It has spread as a weed to all the continents of the world and has adapted to climates from tropical to subarctic. Its adaptation to many agricultural habitats and great reproductive capacity has ranked it as the 16th worst weed in the world. It is a weed seen in 21 crops in more than 30 countries around the world.

Cyperus esculentus, has many other names as nutsedge, yellow nut grass, Zulu nut, earth nut, earth almond, nut grass, rush nuts, yellow nut sedge and chufas. Chufa tubers are daily ingredients of the diet of many people in North Africa and Spain [1]. In North Africa, the tubers are consumed in their natural form or after being soaked in water for some hours. In Spain, the tubers are consumed mainly as a drink called locally « horchata de chufa » (chufa milk). Tiger nut has been cultivated as a livestock food and for human consumption, it can be eaten either a nut or grated, baked or used for ice cream and beverage making, oil extraction and incorporation of components in food products.

In many countries, *Cyperus esculentus* is considered a weed and it is underutilized [2,3]. Tiger nut is not widely used in agriculture; it has been poorly investigated and attracted very little scientific and technological interest, and is not commonly used as a food ingredient which may be due to a number of factors. These may include insufficient information on the nutritional profile of the tuber, oil extraction techniques, usability of the oil and possibly simply an awareness of the potential uses of the tuber [4]. Development of new products from the tubers could enhance more interest in this crop. In this respect, various opportunities are offered: source of dietary fiber, use of its oil in cooking, frying and salad preparation, production of caramel to be used as a food additive and flavouring agent.

Our present study in this review encloses on biological and chemical properties of *Cyperus esculentus*. Complete information regarding this plant has been collected from various books and journals since the last years. This compiled data reflects the therapeutic properties of this herb. This review article is very helpful for researchers to focus on natural plant products to find out new therapeutically important compounds responsible for its claimed traditional importance. Several therapeutically important natural compounds have been isolated (such as alkaloids, flavonoids, carbohydrates, tannins, saponins, and steroids) and they can serve as very potent and reliable drug candidate for treatment of various disorders.

TIGER NUT COMPOSITION

The obtained data for the composition of tiger nut tubers indicated that their moisture content was of 3.75% [5] and it was lower than the moisture contents reported for other tubers [6]. The oil content varies between 22.8 and 32.8 g/100 g [7,8,9,10,11]. The carbohydrate content of tiger nut tubers was found to be the first component which composed of starch and dietary fiber (consists mainly of cellulose and lignin; xylose is present as a substantial proportion of the insoluble dietary fiber fraction). Their profile and relative content change according to tiger nut varieties and ripening stage. Regarding total sugar content, in general, tubers have high contents. When they were compared with those of other tubers and nuts, the sugar level of tiger nut was relatively low. However, the taste of tiger nut depends on the sugar content to give a very characteristic flavor. These researchers concluded that tiger nut could be useful as a good source of dietary fiber in food technology because of its large amount and its pleasant nutty flavor.

Other studies revealed that tiger nut tuber have high calcium, sodium and copper and low magnesium, manganese, phosphorus, iron, zinc and copper mineral contents. The high values of calcium found in the tiger nut, are adequate for bone and teeth development in infants. The presence of other minerals such as iron is highly important because of its requirement for blood formation [12,5].

A total of seventeen amino acids was identified in the tiger nut tubers, namely cysteine (Cys), proline (Pro), L-alanine (Ala), L-aspartic acid (Asp), glycine (Gly), L-glutamine acid (Glu), arginine (Arg) and the essential amino acids : isoleucine (Ile), leucine (Leu), lysine (Lys), L-histidine (His), L-methionine (Met), L-threonine (Thr), L-phenylalanine (Phe), L-tyrosine (Tyr), L-serine (Ser) and L-valine (Val). The amino acids profile was dominated by L-aspartic acid, which resulted from the conversion of asparagine. Other important amino acids were L-glutamine acid, which resulted from glutamine, followed by leucine, L-alanine and Arginine [12,5].

The total lipid content of tiger nut tuber was 17.6% on a dry weight basis. The neutral lipid (90% of the total lipid) was the main lipid component, while glycolipid and phospholipid were present at 6.9 and 3.1%, respectively [13]. In another study, the phospholipid percentage was 5.4% with ethanolamine, inositol, choline and serine glycerophospholipid as the major classes. Choline glycerophospholipid was the most abundant at 33.0% [14].

The fatty acid (FA) profile of the seed oil from tiger nut closely resembles the FA profile for olive oil ; with FA composition of oleic acid C18:1 (65.55%), palmitic C16:0 (16.32%), linoleic acid C18:2 (12.13%), stearic acid C18:0 (5.33%) and arachidic acid C20:0 (0.68%) [15]. The oleic acid content of

tiger nut oil is much greater than that of most other vegetable oils, such as sunflower oil (23.6%), soybean oil (24.9%), or corn oil (23.8%), but comparable to that of olive oil [16,17,18]. Tiger nut oil with it high percentage of oleic acid, should be relatively stable and resistant to oxidation [9].

According to Yeboah et al. [15], the major classes of triacylglycerols (TAGs) in tiger nut oil were C54:3 (29.00%), C52:2 (27.35%) and C54:4 (12.20%). The C54:3 was mainly triolein, in which oleoyl acyl group occupied both the sn-1/3 and the sn-2 positions at 52.68% and 77.62% respectively. It is noting also that occupancy of the sn-2 position in the TAGs of the tiger nut oil was 100% unsaturated, a structural feature which is consistent with most vegetable oils. This structural feature make tiger nut oil particularly nutritive as it ensures easy absorption of unsaturated FAs into the blood stream.

The unsaponifiable matter of tiger nut oil was identified as consisting of hydrocarbons, waxes, triterpene alcohols, sterols esters, higher alcoholic esters and sterols. Hydrocarbons constituted the highest of these, followed by sterols. The hydrocarbon composition however was not given and is not given elsewhere. It will be interesting to discover what the hydrocarbons are and if compounds such as squalene are present.

Other classes of compounds that comprise the unsaponifiable fraction in tiger nut oil include tocopherols, phytosterols, polyphenols and possibly carotenoids. Yeboah et al. [15] have studied the phytosterols and the vitamin E compounds (tocopherols and tocotrienols) present in tiger nut oil. The total vitamin E content was 12.1 µg/g of oil and the phytosterol content was dominated by β-sitosterol at 50.37%, which translated into 517.25 µg/g of oil. The other common vegetable oil phytosterols like stigmasterol (20.62%, 225.25 µg/g of oil) and campesterol (15.33%, 161.35 µg/g of oil) were found to be present in larger amounts. Other minor phytosterols in tiger nut oil were Δ5-avenasterol (3.75%, 17.04 µg/g of oil), which together made a total phytosterol content of 986.49 µg/g of tiger nut oil. This moderately high content of phytosterols further enriches the quality and value of tiger nut oil as a food source. In fact, tiger nut oil is much richer in phytosterols than olive oil (100 µg/g of oil), and hence it is the phytosterol profile of tiger nut oil that truly distinguishes it from olive oil.

Recently, the concentration of total polyphenols in tiger nut oil was quantified and given as 16.5 mg Gallic Acid Equivalent (GAE) per 100 g oil [19]. No study has been performed to identify all the phenolic compounds in tiger nut oil.

The concentration of anti-nutriments (tannins, saponins, phytate, oxalates and cyanogenic glycosides) in raw tiger nut tubers was found to be very low compared to that in nuts such as peanuts [20]. Moreover, anti-nutriments screening showed that a higher content of alkaloids, sterols, and resins than cyanogenic glycosides, saponins, and tannins were detected in raw tiger nut tubers. In the roasted tuber, only alkaloids, sterols, and resins were detected [21]. Hence their concentrations in the oil would be expected to be insignificant.

FOOD APPLICATION

The French chemist Lesant in 1822, the Italian researcher Semmola in 1835 and later the Spanish professor Torres Munoz in 1851 were among the first to analyze the tubers of tiger nut; between 1921 and 1924 Pieraerts published works on the composition of the tubers and the cultivation of tiger nut in the Belgian colonies of that time, in Egypt and other Mediterranean countries, the tubers were consumed after soaking or roasting and were also used as a substitute for coffee and chocolate and the fatty oil extracted from tiger nut tubers was used as a food product.

Nowadays tiger nut is cultivated in Northern Nigeria and Ghana, where it is made into a sweet meat, and Togo, where it is used principally uncooked as a side dish [22]. Umerie & Enebeli [23] reported on the preparation and characterization of caramel from malted tubers of tiger nut. This caramel appeared as brown-black syrup which remained clear in 50% alcohol; it may be used to add body, flavor, or color to certain baked products, non-alcoholic malt beverages and dark beers, and in the production of condiments. Umerie et al. [24] reported on the isolation and characterization of starch from tiger nut tubers. They concluded that it was easily isolated and was suitable for many applications. The starches obtained from tiger nut and rice showed similar properties; the solutions of the starch exhibited a good paste stability, clarity, and adhesive strength.

The tiger nut is also a representative crop of the Spanish Mediterranean Region, where tubers are used to make a beverage called horchata de chufa. The milky-looking aqueous extract has a pleasant and characteristic flavor of vanilla and almonds. The popularity of this drink has recently extended to other countries such as France, Great Britain, Argentina, and United States of America.

Tiger nut milk has been reported to be used in the treatment of flatulence, indigestion, diarrhoea, and dysentery [25], and its starch content presumably provides prebiotic properties for colon bacteria [26]. It has been found also to be good for preventing atherosclerosis, since its consumption can help prevent heart problems and thrombosis and activate blood circulation, mainly because its unsaturated fatty acid content and its arginine is a precursor of nitric oxide which helps the veins to expand [27]. Tiger nut milk can be drunk by diabetics for its content in low-glycemic carbohydrates (mainly starch) and due to its arginine which liberates hormones that produce insulin [26]. It is also a suitable drink for celiac patients, who are not able to tolerate gluten and also for the lactose-intolerant who stay away from cow milk and many dairy foods. It could also be recommended for those who have problems with digestion, flatulence, and diarrhoea because it provides some digestive enzymes like catalase, lipase, and amylase [25,3].

The inherent nutritional and therapeutic advantage of tiger nuts makes it a good alternative source of milk in yoghurt production. In a previous study by Adgidzi [28], efforts led to the production of acceptable beverages and yoghurt-like products from tiger nuts. The beverage products were found to contain a proximate composition of 1.89 and 2.67% protein, 0.92 and 1.33% fat, 0.16 and 0.21% ash, 0.24 and 0.33% crude fiber, 76.86 and 80.27% moisture and 15.96 and 19.15% carbohydrates. Mineral composition (Calcium (Ca), Potassium (K), Sodium (Na), Magnesium (Mg) and Phosphorus (P) per 100g) ranged between 14.90 and 25.60 mg, 6.40 and 8.10 mg, 1.98 and 3.24 mg, 0.046 and 0.054 mg, 0.060 and 0.083 mg respectively. The yoghurt-like products were found to contain Total Titratable Acidity (TTA) of 1.3 to 2.4%, pH 4.2 to 5.3, viscosity at 60rpm 151.1 to 202.3 cP and peroxide value (PV) 8-11 Meq/kg. Mean scores of sensory evaluation of freshly prepared yoghurt-like products ranged between 2.1 to 4.6, 2.4 to 4.1, 2.2 to 4.9 and 2.4 to 4.6 for flavor, consistency, color and overall acceptability respectively. With the nutritional composition of the tiger nut extracts, it was possible to support the growth of lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and yield yoghurt-like products.

According to El-Shenawy et al. [29], the addition of 10 or 20% of tiger nut extract to Buffalo's skim milk and using the conventional starter together with *Bifidobacterium bifidum* as a probiotic bacterial strain, could serve as a good fortification for manufacturing of nutritional and therapeutic yoghurt, since it will not influence the chemical and sensorial properties, instead it will improve the rheological properties of the final product.

Other foods based on tiger nut are prepared by a wide range of recipes and preparation methods. Its application in food technology as a flour was explored. It is a good alternative to many other flour like wheat flour, as it is gluten free and good for people who cannot take gluten in their diet. Furthermore, tiger nut floor does not lose any of its nutritious properties in the milling process. The fine ground tiger nut flour gives baits a smooth, creamy texture and can be incorporated into any mix as base ingredient, allowing baits to retain moisture [30].

The effect of using tiger nut flour to improve the functional properties of gluten free biscuit was explored by Ahmed & Hussein [31]. Corn flour in the biscuit formulation was replaced at three levels, 10, 20 and 30% with tiger nut flour, and prepared biscuits were analyzed for their proximate composition, physical properties, diameter, thickness, color and texture. Incorporation of tiger nut flour resulted in a significant increase in fiber, fat and ash contents and in a decrease in protein and carbohydrate contents. The spread ratio of the biscuits increased significantly by increasing tiger nut flour content, which is considered a desirable quality attribute. Moreover, measurement of baked biscuits texture showed that hardness and resilience value decreased when tiger nut flour content in the biscuit formulation increased. Microscopic observation revealed that they had the most uniform and homogeneous pore distributions. All these attributes positively influenced the technological quality expressed in shape, cross section structure, hardness and surface appearance particularly with the incorporating tiger nut flour at the ratio of 20%.

Dietary fiber as a versatile food component may be considered to possess 2 types of functional properties: physiological and technological. As well, increased consumption of dietary fiber improves serum lipid concentration, lowers blood pressure, improves blood glucose control in diabetes, promotes regularity, aids in weight loss, and appears to improve immune function [32]. Sanchez-Zapata et al. [33] analyzed the utilization of increasing levels (0%, 5%, 10%, and 15%) of tiger nut fiber in the formulation of pork burgers. This work was based on chemical composition, physicochemical data, cooking characteristics, and sensory properties of burgers. Pork burgers made with tiger nut fiber had higher nutritional value (higher fiber content) and better cooking characteristics (higher cooking yield,

better fat retention and moisture retention) than control burgers. Burgers with tiger nut fiber were perceived as less greasy, less juicy, more grainy, and with less meaty flavor than the control; although this perception did not reduce the overall acceptability of the burgers. Thus, tiger nut fiber addition to burgers is a promising and convenient application, since dietary fiber of the product was significantly increased without changes in sensory acceptance. These authors have also analyzed the tiger nut fiber application in other meat products such as a cooked product, bologna sausage [34], and some Spanish dry-cured products such as « sobrasada » [35] and « chorizo » [36] with similar results.

Recently, the effect of the addition of tiger nut fiber as a carrier of unsaturated fatty acids on some parameters in a dry-cured sausage was evaluated [37]. The addition of tiger nut fiber (1% to 2%) improved unsaturated fatty acids incorporation into meat batter: the higher the amount of tiger nut fiber added, the more unsaturated fatty acids were incorporated. Dry-cured sausages with tiger nut fiber and unsaturated fatty acids had higher moisture content than the control, lower pH values, and higher water activity.

Tiger nut liquid co-product is also a valuable source of natural antioxidants (phenolic compounds) with important antioxidant properties (mainly reducing power and inhibition of lipid peroxidation) which also contribute to its suitability as a food ingredient. Sanchez-Zapata et al. [6] analyzed the substitution of water by tiger nut liquid co-product in the formulation of pork burger. Results showed that these formulations had the highest cooking yield, fat and moisture retention, and thickness increase; was observed also a smaller diameter reduction in the pork burgers. Tiger nut liquid co-product addition caused, in the cooked burger, texture improvement, because hardness, gumminess, and chewiness were lower in the samples with tiger nut liquid co-product than in burger formulated with tap water. Thus, utilization of tiger nut liquid co-product, as a substitute for water, in the formulation of pork burgers appears to be a valuable alternative.

PHARMACOLOGICAL ACTIVITIES

Antioxidant Activity

The damage caused by free radicals and/or their resulting oxidative stress on the living cells has been extensively studied in recent years. Free radicals have been demonstrated to be the main initiator for many diseases such as cancer, Alzheimer's disease, Parkinson's disease, and rheumatoid arthritis [38,39]. For this reason, there has been intensive study of the antioxidant properties of plant extract and isolated phytochemicals, with a view to identifying potentially useful antioxidant treatments.

Phytochemicals responsible for antioxidant activities of extracts obtained from *Cyperus esculentus* were investigated by Oloyede et al. [40]. *In vitro* antioxidant activity was determined by three methods, scavenging effect on 2,2-diphenyl-1-picryhydrazyl radical (DPPH), inhibition of hydroxyl radical and peroxide oxidation by ferric thiocynate method. Secondary plant metabolites responsible for observed activities in *Cyperus esculentus* are alkaloids, flavonoids, phenols and glycosides. Hexane and methanol extracts possessed significant antioxidant activity when compared with antioxidant standards, butylated hydroxyl anisole (BHA), ascorbic acid and α-tocopherol used in the assay. The inhibition percentage was between 98.24% and 95.30% at 0.00625 mg/ml for the n-hexane and methanol extracts respectively.

The high antioxidant activity of the *Cyperus esculentus* extracts at low concentration can provide, at least in part, an additional experimental justification for some reported folk medicinal uses of *Cyperus* plants. It shows also that this spice could be very useful for the treatment of ailments resulting from oxidative stress.

Anti Arthritic Activity

For the evaluation of anti-arthritic activity of *Cyperus esculentus*, male Wistar rats grouped in a group of 6 animals each into 4 groups. On day 1 and day 3, they will be injected into the subplantar region of the left hind paw with 0.1 mL of 2% v/v formaldehyde in normal saline. Dosing with standard drug, Diclofenac sodium and extracts will be started on same day and continued for 10 days. Group 1 served as arthritis control, group 2 were Diclofenac sodium treated, group 3 and 4 received 250 and 500 mg/kg of *Cyperus esculentus* oil respectively. The assessment made on the 10th day showed that treatment with *Cyperus esculentus* (500 mg/kg) more significantly reduced ($p<0.01$) the swelling in the injected left hind paw as compared to Diclofenac sodium treated group. On the 10th day the inhibition percentage of paw edema exhibited by *Cyperus esculentus* (500 mg/kg) was 76.58% while Diclofenac sodium treated animals showed maximum inhibition percentage of paw edema 81.37% on the 21th day [41].

Hepatoprotective Activity

Liver diseases are one of the major health problems. Hence the need for the development of

hepatoprotective agents has increased. Many years ago, the tubers of *Cyperus* species have been used as remedy for the treatment of several diseases such as hepatotoxicity [42] and an antioxidative agent [43].
The effect of oral administration of *Cyperus esculentus* oily extract was studied for its hepatoprotective and hepatocurative activities against CCI4-induced hepatic damage in male albino rats. *Cyperus esculentus* significantly lowered the serum levels of Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) at a dose of 200 mg/kg as compared to CCI4-treated animals [44].

The *in vitro* study of the hepatotoxicity, indicates that the IC50 of the essential oil content of *Cyperus esculentus* tubers on monolayers of rat hepatocytes was >1000 µg/mL concentration and exhibited hepatoprotection at 18.5 µg/mL [45].

Ebojele and Ezenwanne [46] studied the possible effect of aqueous extract of *Cyperus esculentus* on some liver functional indices in rabbit. The result revealed significant increase in serum ALT, AST, and ALP concentrations at the end of the experiment. The observed increase in ALT and AST levels can be attributed to the presence of phytochemicals which are also anti-nutrient substances. Phytochemical analysis of *Cyperus esculentus* revealed its content of alkaloids and saponins amongst others. The mechanisms of action of alkaloids and saponins are similar and it involves complexing with cholesterol to form pores in cell membrane bilayers [47,48]. This may have been the possible mechanism by which *Cyperus esculentus* acted on the liver cells to bring about the increase in the level of ALT and AST concentrations. Hence, the increases in alanine and aspartate aminotransferases, which are specific for the liver cells, indicate some level of hepatotoxicity [49]. Thus, it can be noted that although *Cyperus esculentus* is reported to have both nutritional and health benefits, the changes in the serum levels of these liver enzymes suggest that it may have some hepatotoxic properties especially when taken in high doses and for a prolonged period.

Analgesic Activity

For the evaluation of analgesic activity of *Cyperus esculentus* essential oil, Biradar et al. [41] have induced pain by injecting 0.05 mL of 2.5% formalin in distilled water in the subplantar of the right hind paw of rats. Animals received dose of essential oils (250 mg/kg and 500 mg/kg), indomethacin (10 mg/kg) and 1% CMC 30 min prior to injecting formalin. The amount of time spent licking the injected paw was indicative of pain. The number of licking from 0 to 5 min (first phase) and 15 to 30 min (second phase) were counted after injection. These phases corresponded to neurogenic and inflammatory pains, respectively. Results showed that *Cyperus esculentus* essential oil inhibited both, neurogenic and inflammatory pains at $p<0.01$ at dose of 500 mg/kg level whereas lower doses of essential oil significantly $p<0.05$ blocked the inflammatory pains. Indomethacin showed highest activity in blocking inflammatory pains and did not show significant activity in neurogenic pains. *Cyperus esculentus* (500 mg/kg) was found to inhibit the pain resulting from inflammation better than the neurogenic induced pain. This study indicated that *Cyperus esculentus* essential oil has both peripheral and central analgesic properties. Its peripheral analgesic activity was deduced from its inhibitory effects on chemical induced nociceptive stimuli [50]. Formalin test investigated both: drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the last phase [51]. The formalin test is a very useful method for not only assessing antinociceptive drugs but also helping in the elucidation of the action mechanism. The neurogenic phase is probably a direct result of stimulation in the paw and reflects centrally mediated pain with release of substance. While the late phase is due to the release of histamine, serotonin, bradikynin and prostaglandins. *Cyperus esculentus* essential oil was able to block both phases of the formalin response but the effect was more prominent in the second phase. It showed maximum protection against formalin induced writhing followed by other models probably explained the peripheral analgesic potential of *Cyperus esculentus* prostaglandin inhibitory activity.

Anti Inflammatory Activity

Carrageenan is the sulphated polysaccharide obtained from the seaweed, which is widely used phlogistic agent which shows signs and symptoms of inflammation, which can be assessed as increase in paw thickness in mouse as a result of increased inflammation, edema and increased vascular permeation. Inflammation produced by carrageenan is a triphasic response. In the first phase of inflammation, histamine and serotonin like inflammatory mediators are involved which cause the edema and redness. In the second phase, different cytokines and kinins get released in response to the inflammation produced and the mediators already secreted at the localized site. In the third phase, the cyclooxygenase enzyme plays pivotal role and there is production of prostaglandins which induces pain. In Biradar et al. [41] study, treatment with *Cyperus esculentus* significantly ($p<0.01$) reduced the paw

edema from the 2nd hour after carrageenan injection and inhibited the paw thickness at the 3rd and the 4th hour after carrageenan injection which probably suggested that *Cyperus esculentus* inhibit the prostaglandin formation in the third phase of inflammation. Moreover, pretreatment with *Cyperus esculentus* doses showed a dose dependent effect, there was significant activity showed by 500 mg/kg than 250 mg/kg. The determination of inhibition percentage showed that administration of *Cyperus esculentus*-(500 mg/kg) produced a comparable effect with indomethacin-(10 mg/kg) (69.7% and 72.1% respectively) 4 hours after carrageenan injection.

Endometritis was defined as inflammation of the endometrium (uterine lining), the most common symptoms are abdominal distention or swelling, abnormal vaginal discharge, fever, uterine pain. To treat endometritis we may use antibiotic e.g. tetracycline or gentamicin. The extract of *Cyperus esculentus* may have active constituents to inhibit inflammatory process with low toxicity as compared with antibiotics [52]. However, after 7 and 14 days of induction with *Escherichia coli* intrauterine infection revealed presence of inflammatory cells within the endometrial glands and surrounding glands. The treatment with alcoholic extract of *Cyperus esculentus* noticed cellular debris within the glandular endometrium and few inflammatory cells around the endometrial glands as compared with gentamicin treatment which showed degenerating of glandular epithelium and aggregates of inflammatory cells surrounding degenerating glands and presence of hemosiderin around glands [53]. This may regarded to that extract of *Cyperus esculentus* contained alkaloids, saponins and tannins are known to have physiological activities particularly saponins which have been reported to be useful in reducing inflammation and tannin compounds are responsible for preventing and treating urinary tract infections [54].

Antibacterial Activity

To the best of our knowledge, the antibacterial activity of *Cyperus esculentus* is being reported for the first time in 2010 by Biradar et al. The essential oil of *Cyperus esculentus* was used in concentration of 250 mg-0.5 mg/mL and the antimicrobial activity was performed by serial dilution method on Gram positive, Gram negative and fungi viz: *Staphylococcus aureus*, *Staphylococcus albus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Aspergillus* (fungi). *Cyperus esculentus* essential oil showed variable activities against tested bacteria (Gram positive and Gram negative) and fungi. It is resistant to Gram negative bacteria (*Escherichia coli*) at all concentrations. *Pseudomonas aeruginosa* (Gram negative) bacteria are susceptible to *Cyperus esculentus* from concentrations of 16.6-250 mg/mL. *Staphylococcus aureus* showed susceptibility to *Cyperus esculentus* from concentrations of 125-250 mg/mL. *Staphylococcus albus* (Gram positive), *Candida albicans*, *Aspergillus* (fungi) showed susceptibility at all concentrations.

Seukep et al. [55] assessed the *in vitro* antibacterial activities of seven Cameroonian dietary plants (*Sesamum indicum*, *Sesamum radiatum*, *Cinnamomum zeylanicum*, *Corchous olitorius*, *Cyperus esculentus*, *Adansonia digitata*, *Aframomum kayserianum*), against multidrug resistant (MDR) Gram-negative bacteria (*Escherichia coli*, *Enterobacter aerogenes*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Anterobacter cloacae*) using liquid microbroth dilution method. Results indicate that the plant extracts exhibited activities depending of bacteria strains, with minimal inhibitory concentrations (MICs) values ranged from 64 to 1024 µg/mL on the majority of the 27 tested microbial strains. At the tested concentration range (8 to 1024 µg/mL), *Cyperus esculentus* extract exhibited weak activities against a limited number of strains studied, it is active against 18.52% of the tested microorganisms, while the lowest MIC value (64 µg/mL) was recorded with that of *Aframomum kayserianum* against *Enterobacter aerogenes* EA294.

In order to evaluate the medicinal value of notorious sedge weeds, *Cyperus esculentus* was investigated for its phytochemical constituents and antimicrobial properties [56]. Preliminary qualitative phytochemical constituents and *in vitro* antimicrobial activities were evaluated against four fungi species viz: *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium chrysogenum* and *Candida albicans* and three bacteria species viz: *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Two solvents, water and ethanol were used to produce the extracts and screened for their antimicrobial activity. Antimicrobial activity evaluation of extract against the pathogens was carried out at 100 mg/mL concentration by Disc Diffusion Method for fungi and Disc Diffusion and Agar Well Diffusion Methods for bacteria. Results showed that water extract was insignificant on all pathogens except *P. chrysogenum*. While the ethanolic extracts was effective with fairly high action against *A. fumigatus* and *P. chrysogenum* and high action against *S. typhi*. The result was similar to that obtained from the antibiotic control ciprofloxacin. This indicates the high potency of this plant extract. Isolating and

purifying the bioactive compounds may lead to the development of another suitable antibiotic against *S. typhi*. The result of phytochemical screening showed that *Cyperus esculentus* contained high number of variety of phytochemicals: carbohydrates, ketose, sugars, tannins, flavonoids, and steroids. According to the antimicrobial assays, it can be suggested that most of the phytochemicals in *Cyperus esculentus* do not possess bioactive forms except against *S. typhi*. The steroids, carbohydrates and ketose sugars may be responsible for this activity because these compounds are used in the production of phytoalexins in plants. Phytoalexins are antimicrobial substances synthesized by plant that accumulate rapidly at areas of incompatible pathogen infection [57]. The presence of tannins and flavonoids suggests that the class of tannins present in this plant is made up of non hydrolysable or condensed tannins which are effective anti-infective compounds [58].

In vitro evaluation of antibacterial activity of *Cyperus esculentus* tubers against six bacterial species (Four Gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella typhi*. Two Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus*) was performed also by Kiran et al. [59]. Antibacterial activity of *Cyperus esculentus* tubers showed a promising result in aqueous extract, and recorded a significant activity against all the test pathogens tested at 10 to 100 µL concentration using inhibition zone method.

Anti Convulsant Activity

The anticonvulsant property was assessed by ability to protect against Maximal Electroshock (MES) induced convulsions. Rats of either sex were used, they were divided into four groups of 6 rats each group 1 received saline, group 2 received 25 mg/kg of Phenytoin, group 3 received 250 mg/kg of *Cyperus esculentus* oil and group 4 received 500mg/kg of *Cyperus esculentus* oil. Maximal electroshock of 150 mA current for 0.2 seconds administered through ear electrodes to induce convulsions in the control and treated animals [41]. Results show that concentration of 500mg/kg decreases the duration significantly ($p<0.01$), of clonus (12.00±0.73 sec) and stupor (74.20±0.63 sec) phase of MES induced convulsion as compared to control, clonus (15.67±0.66 sec) and stupor (96.00±1.94 sec). Hence, *Cyperus esculentus* is able to decrease the duration of hind limb extension (extensor phase), clonus and also the duration of stupor phase, which indicate that 500 mg/kg doses possess potent anticonvulsant activity against generalized tonic-clonic seizure while 250 mg/kg dose did not show statistically any significant effect in extensor phase as compared to control.

MES induced tonic seizures can be prevented either by drugs that inhibit voltage dependant Na^+ channels such as Phenytoin, Valproate, Felbamate and Lamotrigine or by drugs that block glutaminergic excitation mediated by the n-methyl-D-aspartate (NMDA) receptor, such as Felbamate. Essential oil follows any one of the above mechanism [60].

Atherosclerotic Activity

Atherosclerosis is a pathological process, where continued recruitment of mononuclear cells and proliferation and migration of smooth muscle cells results in the growth of fatty streak lesions into larger fibro-fatty plaques. Apolipoprotein E (ApoE) is an important legend for the uptake of lipoproteins, and deficiency of ApoE leads to the accumulation of low-density lipoprotein (LDL) receptor gene family. A recent study utilizing $ApoE^{-/-}$ mouse, which spontaneously develop atherosclerosis on low-fat chow diets, showed that feeding these mice on a diet supplemented with the whole tubers of *Cyperus esculentus* resulted in attenuation of the development of atherosclerotic lesions [61]. The anti-atherosclerotic effect was associated with a decrease in the number of proinflammatory monocytes ($CD11b^+$ and $CD14^+$), macrophages and dendritic cells ($CD11c^+$) in blood.

This effect is due to the decrease in the activation of LFA-1 as reflected by the decrease in their expression. LFA-1 is upregulated after specific or non specific inflammation to mediate recruitment of inflammatory cells into the inflammatory foci [62]. It has been found recently that statins, which possess potent anti-atherosclerotic effects [63], selectively inhibit LFA-1 binding activities [64].

The impact of feeding with tiger nut on the number and activation of lymphocytes, including $CD4^+$ and $CD8^+$ T cells and B cells was analyzed, investigators [65] found that although feeding $ApoE^{-/-}$ mice diet supplemented with tiger nut slightly decreased $CD8^+$ T cell number, it had no effect on the number of $CD4^+$ T and B lymphocytes. Interestingly however it decreased the expression of IL-2Rα. IL-2Rα is a marker for early T cell activation, thus its expression levels might predict the onset of the inflammatory responses in atherosclerosis.

Further, *in vitro* proliferation of blood and spleen cells from tiger nut-fed $ApoE^{-/-}$ mice showed lower proliferation in responses to ConA and LPS, a T and B cell mitogen, respectively. *In vitro* treatment of blood and spleen cells with water or ethanol extracts of tiger nut markedly increased their proliferation in

responses to ConA. Taken together, these results indicate that ingredients of tiger nut tubers exhibit anti-inflammatory properties upon inflammation, and immunostimulatory effects in immunocompetent hosts.

Protective Effect On Induction Of Sperm Abnormalities

In analogy, lead was shown to implicate testicular lipid peroxidation as evidenced by previous reports [66], this could be due to the formation of free radicals through an exhaustion of antioxidants and subsequently to oxidative stress. On the other hand, since lead does not undergo oxidation-reduction cycle, the effect of lipid on lipid peroxidation is not direct effect, but these changes could rather be due to an indirect effect of lead on the free radical scavenging enzymes and/or glutathione peroxidase. However, the higher membrane lipid content of testes is presumed to make them more vulnerable to oxidative stress. Thereupon, the effect of the extract of *Cyperus esculentus* on the change in sperm count, activity, morphology and testicular histology induces by lead acetate was studied [67]. 18 adult male rats were randomly divided into three experimental groups. Group 1 (control) were given clean drinking water and rat chow. Group 2 were given intraperitoneally injection of 8 mg of lead acetate/ kg body weight, water and rat chow. Group 3 were given intraperitoneally injection of 8 mg of lead acetate/ kg body weight, water with extract of *Cyperus esculentus* and rat chow. The results show in group 2, administered with lead acetate only, a destruction in the testicular histology as abnormal structure of the seminiferous tubules with vacuolar degenerative changes appearing in the cytoplasm of the spermatogenic epithelium and absence of late stage germ cells showed damage and sloughing of somniferous tubules, a decreased sperm count, activity and morphology of sperm head which was observed a high level of abnormality in the sperm head. In the group 3 given extract of *Cyperus esculentus* there was a dose dependent improvement in the induced histopathological changes in the testis of rats caused by effect of lead acetate. These improvements may be either directly on spermatogenesis by reducing lipid peroxidation and prevent or decrease the formation of free radicals by acting as antioxidant, or indirectly through pituitary hypothalamic or sex hormonal effects.

Anti Diarrhoeal Activity

Diarrhoea is a condition that involves the frequent passing of loose or watery stools. Medicinal plants have been used as traditional remedy for diarrhoea for years long and there is renewed interest in the discovery of novel compounds from plant to fight against diarrhoea. Majumder [68] investigated the antidiarrhoeal potential of 70% hydro-ethanolic extract of *Cyperus esculentus* root on castor oil induced diarrhoea in Wister rats. The extract showed dose dependently delayed the onset of diarrhoea and significantly reduced the number of diarrhoeal episodes and the number of animals exhibiting diarrhoea. However, this value was significant at 400 mg/kg dose. The Loperamide, a standard synthetic antidiarrhoeal drug, has shown significant reduction in frequency of defecation and wet faeces. The percentage inhibitions of faecal and small intestinal content with 200 and 400 mg/kg doses of hydro-ethanol extract were 57.73 and 67.21 and also 56.57 and 69.73 respectively. The results point out the presence of some active principles in *Cyperus esculentus* root extract possessing a promising anti-diarrhoeal effect and substantiate the use of this herb as a non specific treatment for diarrhoea in folk medicine.

The presence of flavonoids and tannins are already reported for their anti-diarrhoeal activity. Tannins can evoke an antidiarrhoeal effect and these substances may precipitate proteins of the electrolytes, reduce peristaltic movement and intestinal secretion [69]. The anti-diarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretion which is known to be altered in intestinal condition [70].

CONCLUSION

This review shows that while the chemistry and a few pharmacological aspects of Tiger nut oil have been studied, there are still no strong clinical data available that provide evidence of the efficacy of Tiger nut oil in humans. That Tiger nut oil constituents have pharmacological properties *in vitro* is not sufficient to ascertain the clinical potential of whole Tiger nut oil. More studies are necessary to determine its impact on human health, which should be aimed at demonstrating the intrinsic as well as relative efficacy of this oil compared to other vegetable oils. Interestingly, the position of Tiger nut oil as a natural product with strong consumer expectations resulting from traditional claims of activity that are insufficiently supported by scientific proof is shared by several other plant extracts or products. Such a trend is likely to continue in view of the strong current demand for food supplements. This demand justifies pharmacological studies on these products.

ACKNOWLEDGEMENT

The authors will like to thank staff of Food Science and Nutrition, Physiology and Toxicology

Department (Faculty of Pharmacy of Pamplona) for their scientific contribution and the Tunisian Ministry of High Education, Scientific Research and Technology for supporting this work.

The authors have declared no conflict of interest.

REFERENCES

1. Okladnikov Iu N, Vorkel' Ia B, Trubachev IN, Vlasova NV, Kalacheva GS. Vop Pitan, 1977; 3: 45-48.
2. Ukwuru MU, Ogdobo AC. Pakistan J Nutr, 2011; 10(1): 95–100.
3. Adejuyitan JA. Am J Food Technol, 2011; 6(3): 197-201.
4. Ezeh O, Gordon MH, Niranjan K. Eur J Lipid Sci Technol, 2014; 116: 783-794.
5. Arafat SM, Gaafar AM, Basuny AM, Nassef SL. World Appl Sci J, 2009; 7: 151-156.
6. Sanchez-Zapata E, Fernandez-Lopez J, Pérez-Alarez A. Compr Rev Food Sci Food Saf, 2012; (11): 366-377.
7. Mokady SH, Dolev A. J Sci Food Agric, 1970; 21: 211–4.
8. Addy EO, Eteshola E. J Sci Food Agric, 1984; 35: 437–40.
9. Linssen JPH, Kielman GM, Cozijnsen JL, Pilnik W. Food Chem, 1988; 28: 279-85.
10. Temple VJ, Ojebe TO, Kapu MM. J Sci Food Agric, 1989; 49: 261–2.
11. Coşkuner Y, Ercan R, Karababa E, Nazlican AN. J Sci Food Agric, 2002; 82: 625–31.
12. Oladele AK, Aina JO. Afr J Biotechnol, 2007; 6: 2473-2476.
13. Kim M., No S, Yoon SH. J Am Oil Chem Soc, 2007; 84: 1079-1080.
14. Oderinde RA, Tairu OA. Food Chem, 1992; 45: 279-282.
15. Yeboah SO, Mitei YC, Ngila JC, Wessjohann L, Schmidt J. Food Int Res, 2012; 47: 259-266.
16. Warner K, Knowlton S. J Am Oil Chem Soc, 1997; 74: 1317-1322.
17. Romero A, Cuesta C, Sanchez-Muniz FJ. J Am Oil Chem Soc, 1998; 75: 161-167.
18. Chung J, Choe E. Food Sci Biotechnol, 2001; 10: 446-450.
19. Rehab FMA, El Anany AM. Food Proc Technol, 2012; 3(8): 1-8.
20. Ejigui J, Savoie L, Martin J, Desrosiers T. J Biol Sci, 2005; 5: 597–605.
21. Chukwuma ER, Obioma N, Christopher OI. Pakistan J Nutr, 2010; 9: 709-715.
22. Omode AA, Fatoki OS, Olaogun KA. J Agric Food Chem, 1995; 43: 2850-2853.
23. Umerie SC, Enebeli JN. Bioresour Technol, 1996; 57: 215-216.
24. Umerie SC, Obi NAN, Okafor EO. Bioresour Technol, 1997; 62: 63-65.
25. Bixquert-Jiménez M. Horchata y Salud: Propiedades saludables y de prevención de enfermedades digestivas. In: Fundación Valenciana de Estudios Avanzados, editor. Jornada Chufa y Horchata: Tradición y Salud. Valencia, Spain: Consellería de Agricultura, Pesca y Alimentación: 2003, pp. 71–85.
26. Alegría-Torán A, Farré-Rovira R. Horchata y salud: Aspectos nutricionales y dietéticos. In: Fundación Valenciana de Estudios Avanzados, editor. Jornada Chufa y Horchata: Tradición y Salud. Valencia, Spain: Consellería de Agricultura, Pesca y Alimentación: 2003, pp. 55–70.
27. Martínez-Valls JF. Horchata y Salud: Posibles beneficios de la horchata de chufa en la prevención de la arteriosclerosis. In: Jornada Chufa y Horchata: Tradición y Salud, editor. Fundación Valenciana de Estudios Avanzados. Valencia, Spain: Consellería de Agricultura, Pesca y Alimentación: 2003, pp. 87–94.
28. Adgidzi EA. Effect of processing methods on the yield and quality of aqueous extracts and yoghurt-like products from tiger nuts (*Cyperus esculentus*). MSc Thesis submitted to the department of Food Science and Technology. University of Agriculture, Makurdi, Benue State: 2010, pp 73.
29. El-Shenawy M, Abd El-Aziz M, El-Kholy WI, Fouad MT. J Agric Res Nat Ressour, 2012; 1(2): 20-31.
30. Bamishaiye EI, Bamishaiye OM. Afr J Agric Nutr Dev, 2011; 11 (5): 5157-5170.
31. Ahmed ZS, Hussein AMS. Pol J Food Nutr Sci, 2014; 64(1): 27-33.
32. Anderson JW, Baird P, Davis RH, Ferreri S, Knudtson M, Koraym A. Nutr Rev, 2009; 67: 188–205.
33. Sánchez-Zapata E, Muñoz CM, Fuentes E, Fernández-López J, Sendra E, Sayas E, Navarro C, Pérez-Alvarez JA. Meat Sci, 2010; 85: 70–6.
34. Sanchez-Zapata E, Fuentes-Zaragoza E, Navarro C, Fernandez-Lopez J, Sendra E, Sayas E, Pérez-Alvarez JA. Caracterizacion de un producto carnico cocido, tipo mortadela, con adicion de fibra de chufa D.O. Chufa de Valencia. Proceeding of the V Congreso nacional de Ciencia y Tecnologia de los Alimentos; 2009. Murcia, Spain.
35. Sánchez-Zapata E, Viuda-Martos M, Fuentes-Zaragoza E, Martín-Sánchez A, Fernández-López J, Sendra E, Sayas E, Navarro C, Pérez-Alvarez JA. Effect of tiger nut (*Cyperus esculentus*) fiber addition on Sobrasada

(Spanish dry cured fermented sausage) color properties and quality. Proceedings of International Conference on Food Innovation; 2010. Valencia, Spain.
36. Sánchez-Zapata E, Zunino V, Fuentes-Zaragoza E, Viuda-Martos M, Sayas E, Sendra E, Pérez-Alvarez JA, Fernández-López J. Effect of tiger nut fiber addition on the quality of a Spanish dry-cured pork sausage ("chorizo"). Proceedings of the 5th International Conference on the Quality and Safety in Food Production Chain; 201. Wroclaw, Poland.
37. Sánchez-Zapata E, Díaz-Vela J, Pérez-Chavela ML, Pérez-Alvarez JA, Fernández-López J. Food Bioprocess Technol, 2013; 6(5): 1181-1190.
38. Aruoma OI. J Am Oil Chem Soc, 1998; 75: 199-212.
39. Pulido R, Bravo L, Saura-Calixto F. J Agric Food Chem, 2000; 48(8): 3396-402.
40. Oloyede GK, Abimbade SF, Nwabueze CC. Academia Arena, 2014; 6(1): 77-83.
41. Biradar S, Kangralkar VA, Mandavkar Y, Thakur M, Chougule N. Int J Pharm Pharm Sci, 2010; 2(4): 112-115.
42. Mehta RS, Shankar MB, Geetha M, Saluja AK. Indian J N Prod, 1999; 15(1): 13-17.
43. Satoh A, Yokozawa T, Cho EJ, Okamoto T, Sei Y. Arch Gerontal Geriatr, 2004; 39(1): 69-82.
44. Ameen A, El Eraky WI, Yassin NAZ. J Egypt Soc Pharmacol Exp Ther, 1999; 18(1): 33-41.
45. Hassanein HD, Nazif NM, Aboutabl EA, Hammouda FM. J Appl Sci Res, 2011; 7(12): 2455-2461.
46. Ebojele FO, Ezenwanne EB. Int J Basic Appl Innov Res, 2014; 3(1): 8-13.
47. Wink M. Allelochemical properties of alkaloids. In: The Alkaloids. Vol 43 (G.A. Cordelle d). Academic press, San Diego: 1993, pp. 1-118.
48. Francis G, Zohar K, Harinder PS, Makkar Klaus B. Brit J Nutr, 2002; 88(6): 587-605.
49. Dial SM. Vet Clin, 1995; 25: 257-293.
50. Chen YF, Tsai HY, Wu TS. Planta Med, 1995; 61: 2–8.
51. RaoCh.V, Kartik R, Ojha SK, Amresh G, Rao GMM. In: Experimental animals Hamdard Medicus, 2005; XLVIII: 102–106.
52. Amadi BA, Ibegbulem CO, Egbebu AC. Int J Nat Appl Sci, 2006; 2: 79-81.
53. Hasan HF, Hamzah AM, Zghair Z. J Pharm Clin Sci, 2013; 7: 40-47.
54. Frantisek SS. The natural guide to medicinal herbs and plants. Tiger Barks Cast, Twinkemhan, United Kingdom: 1991, pp. 1-5.
55. Seukep J, Fankam AG, Djeussi DE, Voukeng IK, Tankeo SB, NoumdemJAK, Kuete A. HLN, Kuete V. SpringerPlus, 2013; 2: 363-370.
56. Adeniyi TA, Adeonipekun PA, Omotayo AE. Inter J Trop Med, 2013; 8(4): 92-98.
57. Kodera Y, Ichikawa M, Yoshida J, Kashimoto N, Uda N. Chem Pharm, 2002; 50: 354-363.
58. Okigbo RN, Annagasi CL, Amadi JC. J Med Plants Res, 2009; 3: 86-95.
59. Kiran B, Bhushan S, Lalitha V, Raveesha KA. J Pharm Pharm Sci, 2014; 3(2): 1250-1259.
60. Denizbas IA, Zyazgan SOB, Eskazan E. Gen Pharmacol, 1999; 32: 513–516.
61. Salem ML, Zommara M, Imaizumi K. Am J Immunol, 2005; 1(1): 60-67.
62. Koopman G, Keehnen RM, Lindhout E, Newman W, Shimizu Y, Van Seventer GA. J Immunol, 1994; 152(8): 3760-3767.
63. Ganesh SK, Nass CM, Blumenthal RS. J Cardiovasc Risk, 2003; 10(3): 155-159.
64. Weitz-Schmidt G. Endothelium, 2003; 10(1): 43-47.
65. Zhou X, Stemme S, Hansson GK. Am J Pathol, 1996; 149(2): 359-366.
66. Acharya UR, Acharya S, Mishra M. Ind Health, 2003; 41(3): 291-294.
67. Al-Shaikh MN, Abdul Wahab TAL, Abdul Kareem SH, Hamoudi SR. Inter J Drug Dev Res, 2013; 5 (2): 387-392.
68. Majumder P. Indian J Res Pharm Biotechnol, 2014; 2(3): 1158-1160.
69. Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocete MA, Jiménez J. Plant Medica, 1993; 59: 333-336.
70. Rao VSN, Santos FA, Sobreika TT, Souza MF, Melo LL, Silveria ER. Planta Med, 1997; 63: 146-149.