Investigation of antibacterial activities of *Chrysophyllum albidum* cotyledon and its topical cream formulation

Stella Usifoh[1], Upe Babaiwa[2] Florence Eichie[3], Joy Agbonkonkon[3]
[1]Department of Clinical Pharmacy and Pharmacy Practice, [2]Department of Pharmaceutical Microbiology,
[3]Department of Pharmaceutics and Pharmaceutical Technology, University of Benin, Benin City, Nigeria

Corresponding author: Stella. Usifoh
Email: sfusifoh@uniben.edu; Phone: +234-8056226668

ABSTRACT

Background: Emergence of multidrug resistant bacteria has rekindled interest on plant derived compounds as substitutes for synthetically produced antibacterial agents.

Objective: This study determined the chemical constituent of *Chrysophyllum albidum* seeds, the antibacterial activity of the ethanol extract and its topical cream formulation.

Method: The antimicrobial activity of the seed extract was evaluated against S. aureus, E. coli, *P. aeruginosa and Klebsiella* using agar diffusion method. Chemical constituents were determined with GC-MS.

Results: A solid, pleasant smelling and reddish brown coloured extract with a yield of 1.4%w/v was obtained. The extract inhibited growth of all test bacteria with an inhibitory zone diameter(IZD) of 20.3-22,5mm, while the cream had IZD between 12.5-15mm. GC-MS profile of extract showed the presence of Oleic acid 32.0%, Stearic acid19.86% and Palmitic acid 12.46% among other constituents which amounted to 35.68% of the extract.

Conclusion: The ethanolic extract of *C. albidum* seeds had antibacterial effect against gram positive and gram negative bacteria. 14 chemical compounds including fatty acids; Oleic, Stearic and Palmitic acids have been identified in the seeds. Formulation of extract into cream decreased antibacterial activity, suggestive of the need to increase its concentration in the cream in order to achieve a favourable therapeutic effect.

Keywords: *Chrysophyllum albidum,* anti-bacterial activities*,* gentamicin, topical cream, zone of inhibition.

Etude des activités antibactériennes de *Chrysophyllum albidum cotyledon* et de sa formulation de crème topique

Stella Usifoh[1], Upe Babaiwa[2] Florence Eichie[3], Joy Agbonkonkon[3]
[1]Département de pharmacie clinique et de pratique pharmaceutique, [2] Département de microbiologie pharmaceutique, [3]Département de Pharmacie et technologies pharmaceutiques, Université du Bénin, Benin City, Nigéria

Auteur correspondance : Stella. Usifoh
Email: sfusifoh@uniben.edu; Téléphone: +234-8056226668

RESUME

Contexte : L'émergence de bactéries multi-résistantes a ravivé l'intérêt pour les composés dérivés de plantes en tant que substituts d'agents antibactériens produits par synthèse.

Objectif : Cette étude a déterminé le constituant chimique des graines de *Chrysophyllum albidum*, l'activité antibactérienne de l'extrait d'éthanol et sa formulation de crème topique.

Méthode : L'activité antimicrobienne de l'extrait de graine a été évaluée contre S. aureus, E. coli, *P. aeruginosa* et *Klebsiella* à l'aide de la méthode de diffusion sur gélose. Les constituants chimiques ont été déterminés avec GC-MS.

Résultats : Un extrait solide, à odeur agréable et de couleur brun rougeâtre, avec un rendement de 1,4% p/v a été obtenu. L'extrait a inhibé la croissance de toutes les bactéries testées présentant un diamètre de zone inhibitrice (IZD) de 20,3 à 22,5mm, tandis que la crème avait un IZD compris entre 12,5 et 15mm. Le profil d'extrait GC-MS montrait la présence d'acide oléique à 32,0%, d'acide stéarique à 19,86% et d'acide palmitique à 12,46%, entre autres constituants, qui représentaient 35,68% de l'extrait.

Conclusion : L'extrait éthanolique de graines de *C. albidum* avait un effet antibactérien contre les bactéries à Gram positif et à Gram négatif. 14 composés chimiques, y compris les acides gras ; les acides oléique, stéarique et palmitique ont été identifiés dans les graines. La formulation de l'extrait en crème a diminué l'activité antibactérienne, suggérant la nécessité d'augmenter sa concentration dans la crème afin d'obtenir un effet thérapeutique favorable.

Mots-clés : *Chrysophyllum albidum*, activités antibactériennes, gentamicine, crème topique, zone d'inhibition.

INTRODUCTION

Skin diseases are among the most common health problems with heavy economic burden seen in primary health care settings with a prevalence of 20 – 80% in developing countries.[1] Topical dermatological creams used for prevention, treatment and cosmetic agent include local anesthetics, anti-inflammatory and antimicrobials creams. Skin diseases can be caused by viral, bacteria, fungi, or parasitic agents such as *Staphylococcus aureus*, group A β-hemolytic streptococci and coryneform bacteria. These organisms usually get into systemic circulation through broken skin. Many systemic infections involving skin symptoms are caused either by the pathogen or by toxins like measles, varicella, gonococcemia, and staphylococcal scalded skin syndrome. Dermatophytic fungi have a strong affinity for keratin and invade keratinized tissue of the nails, hair, and skin.[2] The search for drugs that may be effective against such organisms may be obtained from herbs.

Herbal medicine or phytomedicine is the use of a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes.[3] Herbalism has a long tradition of use outside conventional medicine. Herbal drugs have become important in health care as improvements in analysis and quality control, along with advances in clinical research, show the value of herbal medicine in treating and preventing disease.[4] There is increase awareness and general acceptability of the use of herbal drugs in today's medical practice. According to World Health Organization, 70 to 95% of the developing world population rely on herbal medicine for primary care.[5] At least 7,000 medicinal compounds in modern pharmacopoeia are derived from plants,[6] and many showed a positive correlation between their modern therapeutic use and the traditional use of the plants from which they were derived.[7] Herbal medicine has led to the discovery of a number of new drugs, and non-drug substances like food supplements and additives.[8]

Creams are semisolid drug products topically administered on the skin to exert local action. They possess a relatively soft, spreadable consistency formulated as either water-in-oil or oil-in-water emulsions.[9] Creams are multiphase preparations consisting of a lipophilic phase and an aqueous phase. Creams are formed by incorporating water into a water-absorbing ointment.[10]

The plant *Chrysophyllum albidum* (popularly called African star apple and Otien in the local Edo parlance) is a small to medium buttressed tree species, up to 25-37 m in height. *Chrysophyllum albidum* is distributed throughout the southern part of Nigeria.[11, 12]The seeds are about 1-1.5 x 2 cm, beanlike, shiny when ripe, compressed, with one sharp edge and a star-shaped arrangement in the fruit. The seed coats are hard, bony, shiny, and dark brown, and when broken reveal white-coloured cotyledons.[13,14]

The roots, barks, and leaves of *C. albidum* have been employed in folklore medicine. The bark is used for the treatment of yellow and malaria fevers, while the leaf is used for the treatment of skin eruption, stomach ache and diarrhea.[12] C. albidum is one of the staple fruits highly consumed in Southern part of Nigeria when in season. Studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables and it has been suggested that antioxidants found in large quantities in fruits and vegetables may be responsible for this protective effect.[15, 16] To buttress the use of different parts of *C. albidum* in Folk medicine, a study on the antimicrobial constituents of *C. albidum* seed cotyledons reported the isolation and characterization of Eleagnine components and the minimum inhibitory activities of the extract against various microorganisms.[11] Cotyledons from the seeds of *Chrysophyllum albidum* G. Don-Holl. (Sapotaceae), are used in ointments in the treatment of vaginal and dermatological infections in Western Nigeria.[11] However, there is no literature evidence reporting on the antimicrobial activity of the extracted components from *C. albidum* formulated into cream base preparation. Hence the objective of this study was to determine the chemical constituent of *Chrysophyllum albidum* seeds, the antibacterial activity of the ethanol extract and its topical cream formulation.

METHODS

Ripe fruits of *Chrysophyllum albidum* fruits were obtained from a local market Benin City, Edo State, Nigeria. The bacteria species used for the study were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. They were provided by the Department of Pharmaceutical Microbiology, University of Benin, Benin City. Nutrient agar was obtained from Tulip Diagnostics Limited, India, while cetomacrogol emulsifying wax, white soft paraffin and liquid paraffin were obtained from Halewood Chemicals Ltd, England. Ethanol (98%) was obtained from Sigma-Aldrich, Germany. All other chemicals employed in the study were of reagent grades.

Extraction of *Chrysophyllum albidum* seed cotyledons

The seeds of *C. albidum* were air-dried for 48 hours after which the hard shells were removed to obtain the cotyledons. The cotyledons were air-dried for two weeks, and blended into powder, 740g of the powdered cotyledon was dispersed in 1.5L of ethanol. The mixture was macerated and allowed to soak for 48 hours. The extract was filtered using Whatman filter paper 1 and the filtrate was concentrated by evaporation at room temperature for 4 days. The concentrate was stored in an airtight container in a refrigerator for further investigation.

Preparations of *Chrysophyllum albidum* cream

C. albidum extract of different concentrations (150 - 250mg/ml) were incorporated into cetomacrogol ointment BP base with appropriate volume of water to form oil in water aqueous creams. Detailed procedure for the formulation of the aqueous cream is as described by British Pharmacopeia 2004. [17]. In a similar manner, varying concentrations (100 – 250µg/ml) gentamicin were incorporated into cetomacrogol ointment base BP in addition with appropriate volume of water to form oil in water creams

Gas chromatography and Mass spectroscopy (GC-MS) of the extract

The Shimadzu GC-MS QP was used. The sample was analyzed under the same conditions as for reference standards, and in the Selective Ion Mode (SIM) with m/z values ranging from 65 to 500. Split less injection mode was used with injection temperature at 250°C while column oven temperature was ramped between 80 and 280°C. The column ovum temperature program was as follows: 90^0 C, held for 1 min, 12^0 C min^{-1} to 150^0 C, held for 1 min, 2^0C min^{-1} to 230^0C, held for 3 min, 10^0C min^{-1} to 275^0 C, held for 25 min. The GC-MS was operated at a pressure of 79.5 kPa and helium carrier gas flow rate was 1 mL/min. A chromatograph of relative abundance against m/z was obtained and from an electronic library data search, possible structures were elucidated through mass fragmentation pattern.

Determination of antimicrobial activity of *C. albidum* extract, the formulated cream and gentamicin.

Antimicrobial susceptibility test was determined using agar well diffusion method.[18] Standardized inoculums of each test bacteria were streaked on the surface of agar already set and dried MH agar plate (containing 30ml Muller Hinton agar). A sterile cork borer was used to bore six wells in each agar plate. Each well was seeded and filled with 200µL of test antimicrobial agent at concentrations of 130mg/ml and 160mg/ml per well for extract and cream respectively.

Similar procedure was carried out with gentamicin (0.5mg/ml) to give 50µg per well. Negative control (without the extract and standard drug) and positive control (viability test for bacteria used) were carried out for each set of experiment. All plates were incubated in an upright position for 18 – 24 hours at 37°C. The inhibition zone diameters (IZD) were measured in millimeter (mm) as an index of killing or inhibitory action of the test antimicrobial agents against a given bacterium. All experiments were carried out in triplicates.

RESULTS

The *C. albidum* seed gave a solid, pleasant smelling and reddish brown coloured extract with a yield of 1.4%w/v as shown in table 1. In this study all test bacteria were susceptible to inhibitory effect of *C. albidum* extract, the formulated cream and gentamicin cream. A higher inhibitory activity was observed with the crude extract compared to the formulated extract and gentamicin cream as presented in table 2.

GC-MS chromatogram (figure 1) showed several peaks with corresponding retention time. When compared with standard spectral, 14 compounds were identified and presented in table 3. The predominant compounds with antibacterial activity were 9-Octadecanoic acid (Oleic acid) 32.0%, Octadecanoic acid (Stearic acid) 19.86% and Hexadecanoic acid (Palmitic acid) 12.46%

Table 1: Physical properties and yield of *C. albidum* extract and the formulated cream.

Physical properties	Antimicrobial agents	
	***C. albidum* extract**	**Formulated extract cream**
Colour	Reddish brown	Off white
Texture	Solid	Creamy
Odour	Pleasant	Blank
Yield	1.4%w/v	

Table 2: Inhibition zone diameter of extracts against test bacteria

Bacteria	Zone of inhibition (mm) (Mean±SD)		
	***C. albidum* extract (130 mg/ml)**	**Formulated extract cream (160 mg/ml)**	**gentamicin cream (0.5 mg/ml)**
Pseudomonas aeruginosa	22.5 ± 0.7	12.5 ± 0.3	17.8 ± 0.5
Escherichia coli	21.43 ± 0.3	13.8 ± 0.7	19.05 ± 0.5
Klebsiella pneumonia	23.5 ± 0.5	14.5 ± 0.5	18.53± 0.4
Staphylococcus aureus	20.3 ± 0.4	15.4 ± 0.3	18.6 ± 0.3

Gas chromatography and Mass spectroscopy results

Gas chromatography-mass spectroscopy analysis of the pure *Chrysophyllum albidum* cotyledon ethanolic extract was carried out at NARICT, Zaria. The concentrations of the different compounds were determined by using the peak area for the different peaks shown in the chromatogram in Figure 1. A total of fourteen peaks were seen and the major prominent peaks were: 9-octadecenoic acid (46.29%), hexadecanoic acid (22.23%), octadecanoic acid (14.46%) and 9,12-octadecadienoic acid (9.78%) along side with six other trace peaks, as revealed by spectrum comparison of the 14 peaks (lines 1 to 14) to the matching available library.

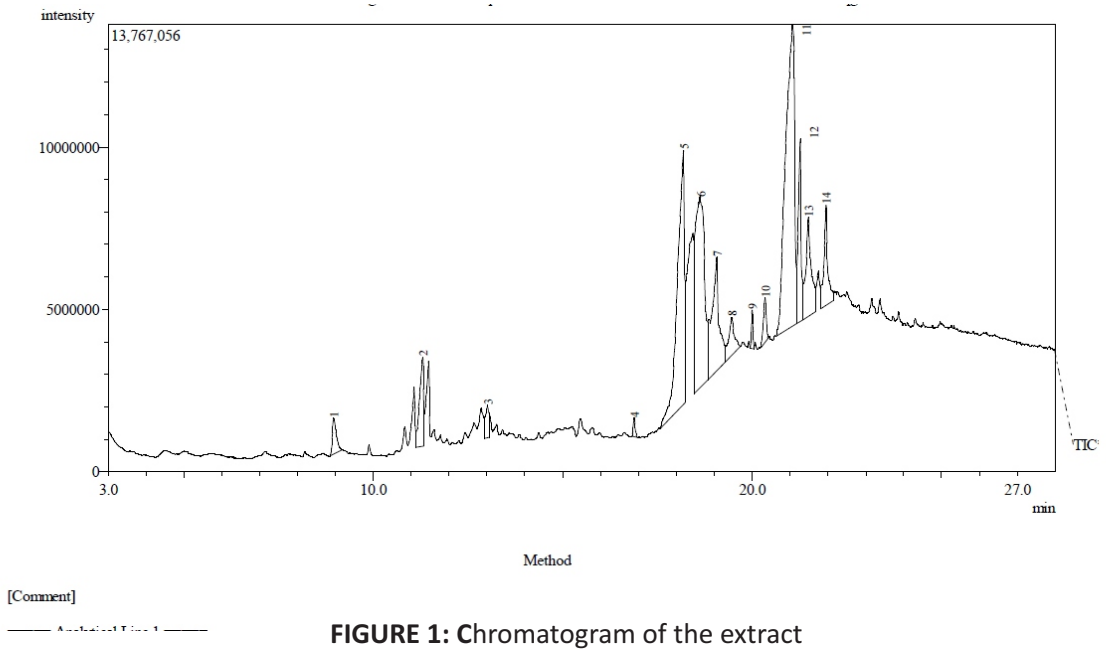

FIGURE 1: Chromatogram of the extract

Table 3: Chemical compounds identified by GC-MC analysis of extract

S/N	IUPAC NAME	%	Retention Time[Minutes}
1	Phenol	2.33	8.96
2	Hexadecanoic acid	5.79	11.31
3	Docosenoic acid	2.13	13.02
4	Decanoic acid	1.21	16.89
5	n- Hexadecanoic acid	16.51	18.19
6	9- Octadecanoic acid	12.46	18.63
7	Octadecanoic aci d	7.35	19.07
8	13 –Octadecatriene	2.47	19.46
9	16 - Octadecenoate	2.49	20.00
10	13 -Octadecatriene	2.96	20.34
11	9-Octadecenoiccid	19.60	21.07
12	Octadecanoic acid	19.86	21.27
13	11,14 - Eicosadienoic acid	6.44	21.49
14	9, 12 -Octadecadienoic acid	6.54	21.95
TOTAL		100	

DISCUSSION

Susceptibility of test bacteria to antimicrobial agents is evident by the presence of growth inhibitory zones on a seeded agar plate. This zone was measured as an index of killing or inhibitory action of the extract against test bacteria. Extracts were considered active at a zone of inhibition diameter of >10mm.[19] This study showed susceptibility of test bacteria to antimicrobial agents as evident by the presence of growth inhibitory zones on a seeded agar plate.

In this study the extract showed high antibacterial activity (IZD, 20-25mm) and the extract cream an intermediate activity IZD between (12.5-15mm) as described by kyung et al 2007.[20] Topical formulation of ethanolic extract into cream markedly decreased antibacterial activity, suggestive of the need to increase the concentration of the extract in the cream formulation to achieve a favorable therapeutic effect. The zone of inhibition indicated sensitivity of the microorganisms to the *Chrysophyllum albidum* extract which is similar to work done by Idowu *et al,* 2003 [11] where the methanolic extract of the cotyledons from the seeds of *Chrysophyllum albidum* led to the isolation and characterisation of eleagnine (1,2,3,4-tetrahydro-1-methyl-β-carboline), tetrahydro-2-methylharman (1,2,3,4-tetrahydro-1, 2 -dimethyl-β-carboline) and skatole (3-methylindole). Eleagnine was the main antimicrobial compound with activity against *Candida albicans* (MIC 1250μg/ml) and *Candida pseudotropicalis* (MIC 250mg/ml). It was also active against *Escherichia coli* (MIC 500mg/ml), *Staphylococcus aureus* (MIC 62.5μg/ml), *Pseudomonas aeruginosa* (MIC 500μg/ml) and *Bacillus subtilis* (MIC 250μg/ml). 1,2,3,4-tetrahydro-1, 2 -dimethyl-β-carboline also showed activity against these organisms while skatole had little activity.[11]

Cotyledons from the seeds of *Chrysophyllum albidum* G. Don-Holl (Sapotaceae), have been used in ointments in the treatment of vaginal and dermatological infections in Western Nigeria.[11] Similarly, other plants also used for skin infections like the leaves of *Cassia alata* in the treatment of *Tinea imbricate in W*estern Pacific [1]

The observed antimicrobial effect of the extract may not be unrelated to the presence of fatty acid with known antimicrobial activity.[21] Some of which were reported in the GS-MS chromatogram obtained for this extract. Presence of Oleic acid 32.0%, Stearic 19.86% and palmitic acid 12.46% among other constituent compounds which accounted for 35.68% of the extract. These fatty acids have been reported to have antibacterial and antifungal action[22] which may be due to ability of fatty acids to intercalate into bacteria cell membrane causing fluidity, permeability changes and consequently the unstable bacteria cell lysis. Subsequent studies should adhere to the extraction procedure in order to get optimal yield and immature fruits should be avoided.

CONCLUSION

Chrysophyllum albidum cotyledons ethanolic extract possessed antimicrobial activity against *Pseudomonas*

aeruginosa, *Staphylococcus aureus* and *Escherichia coli.* Topical formulation of ethanolic extract into cream markedly decreased antibacterial activity, suggestive of the need to increase the concentration of the extract in the cream formulation in order to achieve a favorable therapeutic and comparable antibacterial activity with the standard gentamicin; which could be useful in the treatment and management of dermatological conditions.

ACKNOWLEDGEMENT

The authors are grateful to staff of the Departments of Pharmaceutical Microbiology and Pharmacognosy, Faculty of Pharmacy, University of Benin for all technical assistance

REFERENCES

1. Hay R, Bendeck SE and Chen S. Skin Diseases. In: Jamison DT, Breman JG. Measham AR, Alleyne G, Claeson M, Evans DB, Jha P, Mills A, and Musgrove P (2006). Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development. The World Bank. Chapter 37. Available from: https://www.ncbi.nlm.nih.gov. Co-published by Oxford University Press, New York.
2. Aly R (1996). Microbial Infections of Skin and Nails. In: Baron S. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 98. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8301/
3. "What is herbalism?". Canadian Herbalist Association of British Columbia (2015). http://www.chaofbc.ca/about/what-is-herbalism/. Retrieved 18th March 2018
4. Hawkins EB, Erhlich SD (2007). Herbal Medicine: Overview. Pg 1-4. https://www.umm.edu /health/medical/altmed/treatment/herbal-medicine-00351.html
5. WHO (World Health Organization) (2011). The World Traditional Medicines Situation, in Traditional medicines: Global Situation, Issues and Challenges. Geneva 3:1-14.
6. Interactive European Network for Industrial Crops and their Applications (2000-2005). "Summary Report for the European Union". QLK5-CT-2000-00111. Archived from the original on 2013-12-22. [www.ienica.net/reports/ienicafinalsummaryreport2000-2005.pdf
7. Fabricant DS, Farnsworth NR (March 2001). "The value of plants used in traditional medicine for drug discovery". Environmental Health Perspective. 109 Suppl 1 (Suppl 1): 69-75.
8. Kunle OF, Egharevba HO and Amadu PO (2012). Standardization of Herbal medicines - A Review. International Journal of Biodiversity and Conservation. Vol 4(3): 101-112.
9. Clarence TU, Vinod PS, Kris D, Gary E, Gordon F, Howard M, Margareth M, Howard R, Steve S, Kailas T and Avi Y (2009). Topical and Transdermal Drug Products in: Stimuli to the Revision process. Pharmaceutical Forum. Vol. 35(3). Pp 750-754.
10. Daniels R and Krie U (2007). Galenics of Dermal Products-Vehicles, Properties and Drug release. Journal der Deutchen Dermatologischen Gesellshaft. 5: 367-383.
11. Idowu TO, Onawunmi GO, Ogundaini AO, Adesanya SA (2003). Antimicrobial constituents of *Chrysophyllum albidum* seed cotyledons. Nigerian Journal of Natural Products and Medicine Vol.7 33-36.
12. Idowu TO, Iwalewa EO, Aderogba MA, Akinpelu BA, Ogundaini AO (2006). Biochemical and behavioural effects of eleagnine from *Chrysophyllum albidum*. Journal of Biological Sciences. 6: 1029-1034.
13. Madubuike FN, and Ogbonnaya O (2009). The Potential Use of White Star Apple seed (*Chrysophyllum albidum*) and Physic Nut (Jatropha Curcas) as Feed Ingredients for Rats. Nigeria Agricultural Journal 1:97-105.
14. Adisa SA (2000). Vitamin C, Protein and mineral contents of African Apple (*Chrysophillum albidum*) In: Proceedings of the 18th annual conference of NIST (eds) Garba SA, Ijagbone IF , Iyagba AO Iyamu AO, Kilani AS, Ufaruna N, pp. 141-146.
15. Brurits M and Bucar F (2002). Antioxidant activity of *Chrysophyllum albidum* essential oil. Institute of Pharmacognosy; 14: 323-328.
16. Harnifi H and Amrani S (2007). Review Article Flavonoids as potent Phytochemicals in Cardiovascular Diseases Prevention. Pharmacognosy Review. 2007; 1:193-202
17. British Pharmacopoeia 2004. Published by His Royal majesty, England.
18. Murray PR, Rosenthal KS, Pfallor MA (2009). Medical microbiology, Mosby Inc. 234-239
19. Usman H and Haruna AK (2005). Phytochemical and antimicrobial screenings of the leaf extracts of Celtics Integrifolia. Tropical Bioscience, Vol. 5, (2)

72-76.

20. Kyung KH, WooY, Kim DS, Park HJ and Kim YS (2007). Antimicrobial activity of an edible wild plants, apiifolia virgins bower (clematissapifolia DC). Food Science Biotechnology, Vol. 16:1051-1058.
21. Agoramoorthy G, Chandrasekaran M, Ventatesahi V and Hiu MJ (2007). Antibacterial and antifungi activities of fatty acid methyl esters of the blind your eye mangrove from India. Brazil Journal of Microbiology; 38: 739-742.
22. Ching TH (2008). New Bioactive Fatty acids. Review Article. Asia Pacific Journal of Clinical. Nutrition; 17 (51): 192-195.