THE CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF CHITOSAN-ALGINATE NANOPARTICLES ENCAPSULATING CYMBOPOGON CITRATUS AND CYMBOPOGON NARDUS AQUEOUS EXTRACTS

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Kajian ini bertujuan untuk menghasilkan formulasi nanopartikel polimerik yang bertujuan untuk melingkupi ekstrak akueus dua spesies *Cymbopogon* iaitu *Cymbopogon citratus* (serai makan) dan *Cymbopogon nardus* (serai wangi) yang mengandungi agen antioksidan tidak stabil. Selain daripada mempunyai zarah bersaiz nano yang boleh meningkatkan penembusan pada kulit, nanopartikel juga berkebolehan untuk meningkatkan stabiliti agen terapeutik secara kimia atau fizikal, serta berupaya untuk menyampaikan agen terapeutik ke kawasan yang dikehendaki dalam kandungan konsentrasi yang tinggi. Ekstrak akueus telah diperolehi daripada tiga kaedah ekstrak yang berbeza iaitu merebus, infusi dan 'soxhlet'. Nanopartikel polimerik atau nama khasnya Chitosan-Alginate nanopartikel telah disediakan dengan kaedah gelatin ionik serta dikaji untuk mengemali ciri-ciri nanopartikel seperti saiz, potensi zeta, indeks polydispersity, dan kecekapan pengkapsulan. Semua formulasi juga dinilai untuk aktiviti antioksidan menggunakan kaedah ujian DPPH. Nanopartikel polimerik yang mengandungi *C. nardus* ekstrak akueus oleh kaedah 'soxhlet' didapati memberikan hasil yang memuaskan dari segi saiz zarah, potensi zeta, indeks polydispersity dan kecekapan lingkupan dengan nilai 281±34.31 nm, -36,25±3.43 mV, 0.48 dan 86.9±5%, masing-masing. Semua formulasi nanopartikel ini telah didapati mempunyai aktiviti antioksida, dan salah satunya mempunyai peratus yang tertinggi iaitu 58.29±0.34%.

ABSTRACT

This study aimed to formulate polymeric nanoparticles to encapsulate aqueous extracts of two *Cymbopogon* species which are *Cymopogon citratus* and *Cymbopogon nardus* that contain unstable antioxidant agent. Apart from having the nano-sized particles which may enhance penetration to the skin, nanoparticles are also known for their abilities to increase the stability of therapeutic agents by chemical or physical means, as well as to deliver higher concentrations of drugs to target areas. The aqueous extracts were obtained by three different extraction methods which are decoction, infusion and soxhlet extraction. Polymeric nanoparticles which are chitosan-alginate nanoparticles were prepared by a modified ionic gelation method and characterized for their particle size, zeta potential, polydispersity index, and encapsulation efficiency. All the formulations were also evaluated for the antioxidant activity by free scavenging DPPH assay. The polymeric nanoparticles encapsulating *C. nardus* aqueous extracts by soxhlet extraction were found to give satisfactory results of particle size, zeta potential, polydispersity index and encapsulation efficiency with values of 281±34.31 nm, -36.25±3.43 mV, 0.48 and 86.9±5%, respectively. All the nanoparticles formulations were found out to exhibit antioxidant activity with the highest percentage of inhibition activity of 58.29±0.34%.

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LIST OF SYMBOLS

%	Percentage
^{o}C	Degree celcius
µL	Microliter
mg	Miligram
nm	Nanometer
Abs	Absorbance
cm	Centimeter
v/v	Volume per volume
w/v	Weight per volume
rpm	Rotation per minute
g	Gram
mV	Millivolt
µg	Microgram

LIST OF ABBREVIATIONS

AHA	Alphahydroxy Acids
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
FeCl3	Iron Chloride
FT-IR	Fourier Transform Infrared Spectroscopy
EE	Encapsulation Efficiency
HD	Hydrodistillation
HPLC	High Performance Liuid Chromatography
PCA	Poly(cyanoacrylate)
PDI	Polydispersity Index
PLA	Poly(lactic acid)
PLGA	Poly(D, L-lactide-co-glycolide)
ROS	Reactive Oxygen Species
SCF	Supercritical Fluid Technology
UV	Ultraviolet
UVA	Long Wave Ultraviolet A
UVB	Short Wave Ultraviolet B
UV-VIS	Ultraviolet Visible

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Medicinal plants are the source of good economic value due to its natural medicinal compounds. One of the most commonly known medicinal plants is *Cymbopogon* genus, a member of Gramineae (Poaceae). *Cymbopogon* genus which comprises of 144 species is most commonly known for its uses not only for pharmaceuticals, but also for cosmetics and perfumery applications (Avoseh et al., 2015). In this study, an extended study of *Cymbopogon* species is carried out particularly for two species which are *C. citratus*, also known as normal lemongrass and *C. nardus*, also known as scented lemongrass.

C. Citratus and *C. nardus* extracts contained many chemical components which reside in its essential oil and aqueous extracts such as myrcene, citral, geranial, neral, heptenone, dipentene, limonene, linalool, borneol, geraniol, and citronellol which gives various biological activities (Olorunnisola et al., 2014). These bioactive phytochemical compositions can be obtained from leaf extracts prepared with different solvents such as water, methanol and ethanol (Soares et al., 2013). In traditional medicines, the fresh herbs of *C. citratus* are usually prepared by decoction and infusions while the dried leaves are usually given in the form of tea (Salome et al., 2012). Decoction is suitable for extracting hard plants materials or the compounds which are known to be heat-stable. It is usually resulted in more oil-soluble compounds compared to maceration and infusion (Nn, 2015). In this study, we focus on extraction of decoction, infusion and soxhlet extraction using water as the solvent for the aqueous extract.

C. citratus and *C. nardus* have a wide range of applications due to their antibacterial, antifungal, anti-inflammatory, anti-carcinogenic and antioxidant properties. An antioxidant agent by natural sources such as *C. citratus* and *C. nardus* is highly demanded esppecially for the safety assurance of the consumers. Latest research investigations have proved that antioxidant potential of plant is attributed by the presence of polyphenols, flavonoids, lignins, alkaloids, terpenoids, carotenoids, vitamins and other compounds which have the abilities to remove toxic oxidative products. Phenolic compounds play important role in antioxidant activity as it scavenges reactive oxygen species (ROSs) generated during metabolism in the body such as hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}) and free radicals (Promila & Madan, 2018). The production of free radicals in higher amount than the amount that can be neutralized by the body leads to oxidative stress. Oxidative stress can cause diseases such as cancer, cardiovascular diseases, rheumatoid arthritis, muscle destruction, neurological disorders, inflammatory diseases, and skin aging. Skin aging is caused by the antioxidant capacity that is diminished by ROS along with chronic exposure to Ultraviolet (UV) light (Gianeti & Campos, 2014; Villalobos, 2015). A safe topical application which contains high concentration of active ingredient is required for the efficacy of the treatment. The formulation also needs to have high stability to ensure the efficacy in preventing and eliminating free radicals due to the characteristics of antioxidant which are very unstable due to the fact that it can be oxidized easily. This characteristic makes the formulation ineffective and inactive before reaching the site of action (Leonardi, 2017).

The prevention of the formulation from being ineffectual before reaching the site of action can be achieved by nanoparticles system, specifically Chitosan-Alginate nanoparticles. Chitosan is a cationic polymer which has been widely used in many applications including topical. It is reported to have high potentials not only as a vehicle for controlled delivery, but also for antioxidant and skin protecting agent (Aranaz et al., 2018). Apart from the use of nano-sized particles to provide sustained and controlled release of encapsulated drugs or active ingredients, nanoparticles are also known for their abilities to increase the stability of therapeutic agents by chemical or physical means, and to deliver higher concentrations of drugs to target areas (Goyal et al., 2016).

In this study, polymeric nanoparticle of Chitosan with sodium alginate using the aqueous extracts of *C. citratus* and *C. nardus* is synthesised in order to achieve the objectives of this study. The polymeric nanoparticles have several attractive applications due to their biodegradability, biocompatibility and non-toxic nature (Natrajan et al., 2015). After the formulation of Chitosan-Alginate nanoparticles by using the ionic gelation method, the characterizations of nanoparticles such as particle size, zeta potential, encapsulation efficiency and drug loading are analyzed. For zeta potential, it indicates the stability of the nanoparticles formulation as the magnitude of zeta potential reflecting the degree of electrostatic repulsion between charged particles. Thus, the high degree of zeta potential whether it is negative or positive indicates the resistance of the particles to aggregation, resulting a final stable system (Ahmed & Aljaeid, 2016).

Other than nanoparticles characterizations, antioxidant activity of the Chitosan-Alginate nanoparticles is also analyzed by Free Radical Scavenging DPPH assay which requires the absorbance readings of nanoparticles samples using UV-Vis Spectrophotometer in order to calculate the percentage of scavenging activity. Higher concentration of samples contains higher antioxidant activity, resulting in a lower absorbance reading and higher percentage of scavenging activity. In terms of visual observation, the colour change of DPPH solution from purple to yellow when it is fully reacted with the antioxidant agent in the samples indicates a reduction of free radicals by the antioxidant agent (Jothy et al., 2011).

REFERENCES

Abdullahi, M., Inuwa, B., Umaru, S. M., Adam, S. I., & Khalid, M. A. (2016). Effect of different extraction method on yield and composition of essential oil from Lemongrass (Cymbopogon Citratus) & Eucalyptus Citriodora leaves. *Asian Journal of Biochmical and Pharmaceutical Research*, *6*(2), 181–188. ISSN: 2231-2560

Abhilash, M. (2010). Potential applications of nanoparticles potential applications of nanoparticles. *International Journal of Pharma and Bio Sciences 1*(1), 1-14.

Ahmed, T. A., & Aljaeid, B. M. (2016). Preparation, characterization, and potential application of Chitosan, Chitosan derivatives, and Chitosan metal nanoparticles in pharmaceutical drug delivery. *Drug Design, Development and Therapy, 2016*(10), 483-507. doi: 10.2147/DDDT.S99651

Akhila, A.,(2010). Essential oil - bearing grasses the genus Cymbopogon. *Medicinal and Aromatic Plants - Industrial Profiles*

Altuntas, E., & Yener, G. (2015). Anti-aging potential of a cream containing herbal oils and honey: formulation and in vivo evaluation of effectiveness using non-invasive biophysical techniques. *IOSR Journal of Pharmacy and Biological Sciences Ver. I*, *10*(6), 2319–7676. doi: 10.9790/3008-10615160

Aranaz, I., Acosta, N., Civera, C., Elorza, B., Mingo, J., Castro, C., Gandia, M. D. L., & Caballero, A. H. (2018). Cosmetics and cosmeceutical applications of Chitin, Chitosan and their derivatives. *Polymers 2018, 10*. doi: 10.3390/polym10020213

Avoseh, O., Oyedeji, O., Rungqu, P., Nkeh-Chungag, B., & Oyedeji, A. (2015). Cymbopogon species; ethnopharmacology, phytochemistry and the pharmacological importance. *Molecules*, *20*(5), 7438–7453. doi: 10.3390/molecules20057438

Bhatia, S. (2016). Nanoparticles types, classification, characterization, fabrication methods. *Natural Polymer Drug Delivery Systems*. doi: 10.1007/978-3-319-41129-3

Costa, G., Pinto, J., Vitorino, C., Eugénia, M., José, J., Vitória, I., & Teresa, M. (2016). Polyphenols from Cymbopogon citratus leaves as topical anti-inflammatory agents. *Journal of Ethnopharmacology*, *178*, 222–228. doi: 10.1016/j.jep.2015.12.016

Ekpenyong, C. E., Akpan, E., & Nyoh, A. (2015). Ethnopharmacology, phytochemistry, and biological activities of Cymbopogon citratus (DC) Stapf extracts. *Chinese Journal of Natural Medicines*, *13*(5), 321–337. doi: 10.1016/S1875-5364(15)30023-6

Eltahir, A. S., & Abuereish, B. I. (2010). Leaf and stem anatomy of Cymbopogon citratus and Cymbopogon schoenanthus in Sudan. *Journal of Chemical and Pharmaceutical Research*, *2*(4), 766–771. ISSN 0975-7384

Fabiana, F. L., Pumariega, M. G., Gonz, M., & Tamayo, M. V., Menck, C. F. M., & Lamar, A. S. (2017). Toxic evaluation of Cymbopogon citratus chemical. *Cosmetics 2017 4*(20), 1–5. doi: 10.3390/cosmetics4020020

Gaonkar, R. H., Ganguly, S., Dewanjee, S., Sinha, S., Gupta, A., Ganguly, S., Debnath, M. (2017). Garcinol loaded Vitamin E TPGS emulsified PLGA nanoparticles: preparation, physicochemical characterization, in vitro and in vivo studies. *Scientific Reports*, *7*(1), 1–14. doi: 10.1038/s41598-017-00696-6

Garg, A., Visht, S., Sharma, P. K., & Kumar, N. (2011). Formulation, characterization and application on nanoparticle: a review. *Pelagia Research Library*, *2*(2), 17–26. ISSN 0976-8688

Gianeti, M. D., & Campos, P. M. B. G. M. (2014). Efficacy evaluation of a multifunctional cosmetic formulation: the benefits of a combination of active antioxidant substances. *Molecules 2014*, *19*, 18268–18282. doi: 10.3390/molecules191118268

Gomes, T., Fonseca, P., Azevedo, L. F., Guerra, L., Cristina, I., Moraes, C. De, Watson, D. G. (2016). Polymeric nanoparticles of Brazilian red propolis extract : preparation, characterization , antioxidant and leishmanicidal activity. *Nanoscale Research Letters*, *11*(301), 3-16. doi: 10.1186/s11671-016-1517-3

Goyal, R., Macri, L. K., Kaplan, H. M., & Kohn, J. (2016). Nanoparticles and nanofibers for topical drug delivery. Journal Control Release, 2(240), 77-92. doi: 10.1016/j.jconrel.2015.10.049

Hacke, A. C. M., Marques, J. A., Vellosa, J. C. R., Boligon, A. A., Silva, F. D., Bonini,

J. S., Rocha, J. B. T., Pereira, R. P. (2018). Ethyl acetate fraction of Cymbopogon citratus as a potential source of antioxidant compounds. New Journal Chem. doi: 10.1039/c7nj04352j

Haffiz, J., Norhayati, I., Getha, K., Nor Azah, M. A., Mohd Ilham, A., Lili Sahira, H., Muhd Syamil, A. (2013). Chemical composition and in vitro antitrypanosomal activity of fractions of essential oil from Cymbopogon nardus L. *Tropical Biomedicine*, *30*(1), 9–14.

Hamzah, M. H., Man, H. C., Abidin, Z. Z., & Jamaludin, H., (2014). Comparison of Citronella oil extraction methods from Cymbopogon Nardus grass by ohmic-heated hydrodistillation, hydrodistillation, and steam distillation. *Bioresources*, *9*(1), 256–272.

Janet, D., & Melching, S. (2006). Environmental health criteria 235 dermal absorption. Hanover, Germany: World Health Organization 2006.

Jothy, S. L., Zuraini, Z., & Sasidharan, S. (2011). Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of Cassia fistula seeds extract. *Journal of Medicinal Plants Research*, *5*(10), 1941–1947. ISSN: 1996-0875

Joy, P. P., Skaria, B. P., Mathew, G., Joseph, A., & Sreevidya. (2016). Lemongrass. *Kerala Agricultural University Aromatic and Medicinal Plants Research Station*. doi: 10.13140/RG.2.1.3722.6485

Jumepaeng, T., Prachakool, S., Luthria, D. L., & Chanthai, S. (2013). Determination of antioxidant capacity and α-amylase inhibitory activity of the essential oils from Citronella grass and Lemongrass. *International Food Research Journal*, *20*(1), 481–485.

Kesente, M., Kavetsou, E., Roussaki, M., Blidi, S., Loupassaki, S., Chanioti, S., Siamandoura, P., Stamatogianni, C., Philippou, E., Papaspyrides, C., Vouyiouka, S., & Detsi, A. (2017). Encapsulation of olive leaves extracts in biodegradable pla nanoparticles for use in cosmetic formulation. *Bioengineering 2017*, *4*(75). doi: 10.3390/bioengineering4030075

Kharia, A. A., Singhai, A. K., & Verma, R. (2012). Formulation and evaluation of polymeric nanoparticles of an antiviral drug for gastroretention. *International Journal of Pharmaceutical Sciences and Nanotechnology, 4*(4), 1557-1562.

Lawrence, R., Lawrence, K., Srivastava, R., & Gupta, D. (2015). Antioxidant activity of lemon grass essential oil (Cympopogon citratus) grown in North Indian plains, *The Scientific Temper, 5*, 23-29. ISSN 09768653.

Leonardi, G. R., Silva, S. A. M., Kohn, B. M. (2017). An overview about oxidation in clinical practice of skin aging. *An Bras Dermatol. 2017, 92*(3), 367–374. doi: 10.1590/abd1806-4841.20175481

Lin, C. W., Yu, C. W., Wu, S. C., & Yih, K. H. (2009). DPPH free-radical scavenging activity, total phenolic contents and chemical composition analysis of forty-two kinds of essential oils. *Journal of Food and Drug Analysis, 17*(5), 386–395. doi: 10.1111/j.1468-2494.2009.00531_5.x

Maffei, M. (1990). Essential oils. *Biorenewables Education Laboratory Summer Academy, 5*, 49–52.

Mamillapalli, V., Atmakuri, A. M., & Khantamneni, P. (2016). Nanoparticles for herbal extracts. *Asian Journal of Pharmaceutics, 10*(2), 54–60.

Mukherjee, P. K., Maity, N., Nema, N. K., & Sarkar, B. K. (2011). Phytomedicine Bioactive compounds from natural resources against skin aging. *European Journal of Integrative Medicine*, *19*(1), 64–73. doi: 10.1016/j.phymed.2011.10.003

Nagavarma, B. V. N., Yadav, H. K. S., Ayaz, A., Vasudha, L. S., & Shivakumar, H. G. (2012). Different techniques for preparation of polymeric nanoparticles - a review. *Asian Journal of Pharmaceutical and Clinical Researc*h, 5(3), 16-23. ISSN 0974-2441

Natrajan, D., Srinivasan, S., Sundar, K., & Ravindran, A. (2015). Formulation of essential oil-loaded chitosan-alginate nanocapsules. *Journal of Food and Drug Analysis, 23*(3), 560–568. doi: 10.1016/j.jfda.2015.01.001

Ng, W. L., Yeap, S. K., Bakar, N. S. M. A., Jaafar, W. N. W. M., & Tan, S. G. (2016).

Multiplex PCR assays for species discrimination of Cymbopogon citratus (DC.) Stapf and C. nardus (L.) rendle, two common “serai” (lemon grass) species in Peninsular Malaysia. *Sains Malaysiana*, *45*(3), 323–327. doi: 10.17576/jsm-2016-4503-01

Nitangsam, N. (2012). Monographs of Cymbopogon nardus (L .) rendle and Citronella oil. *Prince of Songkla University*, 1-113.

Nn, A. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants 4*(3), 3–8. doi: 10.4172/2167-0412.1000196

Olorunnisola, S. K., Asiyanbi, H. T., Hammed, A. M., & Simsek, S. (2014). Biological properties of Lemongrass: an overview. *International Food Research Journal*, *21*(2), 455–462.

Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry, 2*(5), 115-119. ISSN: 2278-4136

Promila., & Madan, VK. (2018). A review on the phytochemistry and pharmacology of Cymbopogon citratus Stapf . (Lemongrass). *The Pharma Innovation Journal, 7*(3), 300–304. ISSN 22777695

Ramos-e-Silva, M., Celem, L. R., Ramos-e-Silva, S., & Fucci-da-Costa, A. P. (2013). Anti-aging cosmetics: facts and controversies. *Clinics in Dermatology*, *31*(6), 750–758. doi: 10.1016/j.clindermatol.2013.05.013

Rangari, A. T., & Ravikumar, P. (2015). Polymeric nanoparticles based topical drug delivery: an overview. *Asian Journal of Biomedical and Pharmaceutical Sciences, 5*(47), 5–12. doi: 10.15272/ajbps.v5i47.718

Rahaiee, S., Shojaosadati, S. A., Hashemi, M., Moini, S., & Razavi, S. H. (2015). Improvement of crocin stability by biodegradable nanoparticles of chitosan-alginate. *International Journal of Biological Macromolecules, 79,* 423-432. doi: 10.1016/j.ijbiomac.2015.04.041

Reyes-Jurado, F., Franco-Vega, A., Ramírez-Corona, N., Palou, E., & López-Malo, A. (2015). Essential oils: antimicrobial activities, extraction methods, and their modeling. *Food Engineering Reviews*, *7*(3), 275–297. doi: 10.1007/s12393-014-9099-2

Ruela, A. L. M., Perissinato, A. G., Lino, D. S., & Mudrik, P. S. (2016). Evaluation of skin absorption of drugs from topical and transdermal formulations. *Brazillian Journal of Pharmaceutical Science*, *52*(3), 527-544. doi: 10.1590/S1984-82502016000300018

Sagradas, J., Costa, G., Figueirinha, A., Castel-branco, M. M., Manuel, A., Cabrita, S., Teresa, M. (2015). Gastroprotective effect of Cymbopogon citratus infusion on acute ethanol-induced gastric lesions in rats. *Journal of Ethnopharmacology*, *173*, 134–138. doi: 10.1016/j.jep.2015.07.001

Salome, A. C., Emeka, C. U. C., Ikechukwu, V. O., Brown, A., Calister, E. U., & Godswill, C. O. (2012). Formulation and evaluation of Cymbopogon citratus dried leaf-powder tablets, *6*(48), 3274–3279. doi: 10.5897/AJPP12.575

Scarci, F., & Mailland, F. (2014). In vitro evaluations for a new topical anti-aging formulation. *Journal of Cosmetics Dermatological Sciences and Applications*, *4*(4), 316–322. doi: 10.4236/jcdsa.2014.45041

Soares, M. O., Alves, R. C., Pires, P. C., Oliveira, M. B. P. P., & Vinha, A. F. (2013). Angolan Cymbopogon citratus used for therapeutic benefits : nutritional composition and influence of solvents in phytochemicals content and antioxidant activity of leaf extracts. *Food and Chemical Toxicology*, *60*, 413–418. doi: 10.1016/j.fct.2013.07.064

Subramanian, P., Takwa, C. W. I. C. W., Zubair, N. E. A. (2015). Chemical composition and antibacterial activity of essential oil of Cymbopogon citratus and Cymbopogon nardus against Enterococcus faecalis. *International Journal of Biosciences (IJB)*, *6*(9), 9–17. doi: 10.12692/ijb/6.9.9-17

Suryawanshi, M. A., Mane, V. B., & Kumbhar, G. B. (2016). Methodology to extract essential oils from Lemongrass leaves : solvent extraction approach. *International*

*Research Journal of Engineering and Technol*ogy (IRJET), *3*(8), 1775–1780. ISSN 23950056

Srivastava, V., Dubey, S., & Mishra, A. (2013). A review on Lemon Grass: agricultural and medicinal aspect. *International Research Journal of Pharmacy*, *4*(8), 42–44. doi: 10.7897/2230-8407.04807

Villalobos, M. C. (2015a). Antioxidant activity and citral content of different tea preparations of the above-ground parts of Lemongrass (Cymbopogon citratus Stapf).

Wei, L. S., & Wee, W. (2013). Chemical composition and antimicrobial activity of Cymbopogon nardus Citronella essential oil against systemic bacteria of aquatic animals, *5*(2), 147–152. doi: 10.12692/ijb/6.9.9-17

Wu, X., & Guy, R. H. (2009). Applications of nanoparticles in topical drug delivery and in cosmetics. *Drug Del. Sci. Tech.*, *19*(6) 371-384. doi: 10.1016/S1773-2247(09)50080-9

Yapar, E. A., Inal, O., Erdal, M. S. (2013). Design and in vivo evaluation of emulgel formulations including green tea extract and rose oil. *Acta Pharm. 63*, 531–543. doi: 102478/acph-2013-0037

Yoewono, J. R., Purwanto, R. O., Woolley, C. L., Chang, C., & Shih, W. (2016). Antioxidant activities and oral toxicity studies of Chamaecyparis formosensis and Cymbopogon nardus essential oils. *International Journal of Advanced Scientific Research and Management*, *1*(9), 47-58. ISSN 2455-6378