CHAPTER 8- APPENDIX

8.1 PUBLISHED RESEARCH ARTICLES:

Research Article no. 1: Centum Journal, Published by Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan.

ORIGINAL RESEARCH ARTICLE

Study of sunscreen activity of *Manilkara zapota* (L.) hydro-alcoholic leaf extract and its potentiation using lemongrass oil.

By Reheloo Norma L
By Bhaleroo Suhasini S.

ABSTRACT:
The present study aimed to evaluate the photoabsorptive or sunscreen activity of the hydro-alcoholic extract obtained from the leaves of *Manilkara zapota* (L.) Royen, Sapotaceae, in the ultraviolet (UV) region (200-400 nm) alone, its combination with Lemongrass oil, and its comparison with a standard sunscreen Benzophenone and Para amino benzoic acid (PABA). The extract was prepared by maceration technique using a mixture of distilled water and ethanol (3:7). Methodology included the measurement of effective absorption spectra of the test extract and the standard sunscreens using UV-visible spectrophotometer in the UV range. Based on the preliminary research conducted the hydro-alcoholic extract of *Manilkara zapota* (L) leaves and lemongrass oil were found to possess a potential sunscreen activity by exhibiting highly effective absorption spectra, thereby photoprotection in the UV B and UV C regions and moderately effective in UV A region. Combination of the hydro-alcoholic leaves extract and lemongrass oil exhibited a bathochromic shift in the λmax leading to the enhancement in the effective absorption spectra in the UV A region. The known standard sunscreens benzophenone and para amino benzoic acid (PABA) exhibited its good protection action in the UV B and UV C regions with least effectiveness in the UV A region. The findings thereby conclude the potentiation of sunscreen activity of the hydro-alcoholic extract of *Manilkara zapota* (L.) Royen, leaves using lemongrass oil. Also herbal extract could evolve as a better sunscreen agent as it broadens the UV protection ability of the sunscreens.

KEY WORDS: Sunscreen, *Manilkara zapota* (L.) Royen, hydro -alcoholic, effective absorption spectra, lemongrass oil.

Introduction:
Ultraviolet radiations (UVR) are electromagnetic radiations with wavelength in the range of 200 nm - 400 nm, and are divided into UV A (320-400nm), UV B (290-320 nm) and UV C (100-290 nm). Prolong exposure to UVR has pronounced acute and chronic effects on the skin. Sunburn (erythema) is the well known acute effect. Over the long term exposure,
Formulation, characterization and evaluation of pharmaceutical dosage forms comprising Manilkara zapota extracts.
ABSTRACT:

Acne vulgaris is a common human skin disease, characterized by formation of seborrhea, comedone, inflammatory lesions and presence of an aerobic bacteria *Staphylococcus epidermidis* in the follicular canal and sebum production. Oral and topical antibiotics are the largely prescribed drugs to combat the condition. However bacteria have the ability to develop resistance following repeated or subclinical doses. Hence there is a continual need for newer anti-acne drug molecules for improved drug therapy. Thus the present study aimed to evaluate the hydroalcoholic extract obtained from the bark of *Manilkara zapota* (L.) Royen, Sapotaceae against *S.epidermidis* and its potentiation using lemongrass oil. *In vitro* anti-acne activity using cup plate method and agar dilution method was used to determine the growth inhibition of the organism by the combination of the extract. The results revealed a moderate anti-acne activity of the *M. zapota* bark extract, however incorporation of the lemongrass oil lead to potentiation of the anti-acne activity by enhancing the growth inhibition against *S. epidermidis* compared to the standard antibiotics. The study thus concludes that the herbal combination like *M. zapota* bark extract and lemongrass oil could be used to develop a potential newer anti-acne formulation.

Formulation, characterization and evaluation of pharmaceutical dosage forms comprising *Manilkara zapota* extracts.

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Additional comments if any:

**DR. L. M. DAN**

Name of Observer / Judge: [Signature]

Chintamani, Centum-ISBN2231-1475
IN VITRO PHOTOTOXICITY SCREENING OF HYDROALCOHOLIC EXTRACT OBTAINED FROM MANILKARA ZAPOTA (L) ROYEN LEAVES

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ABSTRACT
Objective: Cosmetic products like sunscreens are being used widely nowadays due to increasing consumer awareness about the harmful effects of ultraviolet light exposure. Conventionally used chemical filters lack photostability hence produce skin reactions. The present research thus aims to evaluate the photostability of the hydro-alcoholic extract obtained from the leaves of Manilkara zapota (L) Royen. Sapotaceae, a potential sunscreen using in vitro phototoxicity assays.

Methods: Leaves of the plant was extracted using hydro-alcoholic solvent (water: ethanol, 3:7) by maceration technique. Candida albicans lawn seeded Sabouraud dextrose agar plate, impregnated with test extract soaked filter paper disc was irradiated at a dose of 0.2 mW/cm² for 4 hours. Absorption spectra of known concentration of the test extract at initial as well as irradiation was measured and compared with standards. Linoleic acid peroxidation test was used to determine the extent of peroxidation formed using TBARS assay.

Results: Candida yeast test revealed that there was no photodegradiation metabolites of the extract formed on irradiation thus growth of the C. albicans remained unaffected. Spectra measurement studies revealed no significant change in the spectrum in the wavelength 250-320 nm of M. zapota extract however the standard phototoxic compound showed a significant change. Linoleic acid peroxidation test revealed a very limited peroxide formation 6.47 μmol/mL comparable with the standard Quecetin. Gallic acid and marketed sunscreen formulation.

Conclusion: All the in vitro phototoxicity assays concluded that the hydroalcoholic extract obtained from Manilkara zapota (L) Royen leaves exhibited photostability to the Ultraviolet irradiation.

Keywords: Manilkara zapota (L), Royen, Hydro-alcoholic, UV radiation, Phototoxicity, Candida yeast, Lipid peroxidation.

INTRODUCTION
Cosmetic Industry is blooming day by day due to constant consumer demand. One of the widely used products is the sunscreens. These products prevent or minimize the harmful effects of the solar radiation on the skin. Ultraviolet radiations (UVR) or solar radiations are electromagnetic radiations with wavelengths in the range of 300 nm - 400 nm. Prolong exposure to UVR has pronounced acute and chronic effects on the skin. Over the long term exposure, degenerative changes in the skin cells, fibrous tissues, blood vessels, leading to premature aging, photodermatoses, inflammation, in most serious cases skin cancers could also erupt [1]. The conventionally used sunscreens have been released nowadays with respect to photostability; major concern considering overexposure to UVR as cause of global warming. Photo instability leads to phototoxic reactions, wherein the photo labile molecule absorbs energy from UV light and releases it into the skin. Further phototoxic response occurs either by formation of photo adducts with biomolecules or through generation of reactive oxygen species (ROS), including singlet oxygen (1O2) and superoxide (O2-) which selectively modify various oxidative reaction mechanisms [2]. Several adverse reactions have been reported during the last decades against chemical sunscreen agents. Cinnamate derivatives (Octyl-Methoxycinnamate), one of the most widely used chemical ultraviolet B (UVB) filter in sunscreen products have shown undesirable reactions such as allergy, photodergy, reactive oxygen species production and estrogenic activities [3], in addition to the external insult the skin also has to cope with endogenous ROS and other free radicals produced during physiological cellular metabolism. Although synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in processed foods and cosmetics, they are reported to have undesirable effects on body cells and are in controversies since last three decades [4, 5]. This may explain the interest in examining plant extracts as a source of natural, cheap, safe and effective antioxidant sunscreens.

Manilkara zapota (L) Royen also called as zapota, or chikoo plant belongs to the family Sapotaceae. It is an evergreen, glabrous tree, growing upto 9-15 m in height; it is cultivated throughout India (most extensive in coastal India such as Maharashtra, Gujarat, Andhra Pradesh, Madras and Bengal States), although being native to Mexico and Central America. Antimicrobial [6] and antioxidant activities are also reported using the ethanolic extracts of leaves [7], seeds [8] and bark [9]. Alcoholic and acetone extract of M. zapota (L) Royen leaves have been reported to reveal the presence of flavonoids as well as phenolic compounds like apigenin-7-O-β-D-glucoside, myricetin-3-O-α-L-rhamnose, and caffeic acid [10]. Formulations of sunscreens mostly contain compounds which are effective UV light absorbers or having photostabilizing properties. Flavonoids are one such constituent and natural substances extracted from plants like green tea polyphenols, Aloe vera leaf extract and aromatic compounds isolated from licorice have been considered as potential sunscreen resources on similar grounds [11]. Ethanolic extract of M. zapota leaves also possess antioxidant activity due to the presence of flavonoids and polyphenolic constituents [12,7]. Although sunscreen activity of the hydroalcoholic extract of M. zapota leaves have been determined, its photostability is yet to be determined. Thus the present research aimed to evaluate the photostability of the M. zapota leaves extract using simple in vitro phototoxicity assays.

MATERIALS AND METHODS
Plant material
Fresh leaves of Manilkara zapota (L) Royen Sapotaceae, were collected from the local garden of Palghar (St. John Technical Campus) District Thane, Maharashtra, India. Leaves of the plant were separated, cleaned, shade dried, coarsely ground and stored in air tight containers. The plant was further authenticated by Dr. Harshad Pandit, Head of the Botany Department, G. N. Khalsa College of Arts, Science and Commerce, Matunga, Mumbai and the specimen no. no. 850554 is deposited in the herbarium of the Department.

Formulation, characterization and evaluation of pharmaceutical dosage forms comprising Manilkara zapota extracts.
Potentiation of Anti-acne activity of hydroalcoholic extract of Manilkara zapota bark against Staphylococcus epidermidis using Lemongrass oil.

Norma Rebello \textsuperscript{1,2*}, Suhasini Bhalerao

Presented a Paper Titled “Potentiation of Anti-acne activity of hydroalcoholic extract of Manilkara zapota bark against Staphylococcus epidermidis using Lemongrass oil” at one day International Research Conference on “Emerging Trends and Innovations in Science and Engineering Education”, organized by ARMIET’s Alamuri Ratnamala Institute of Engineering and Technology, Asangaon, conjointly with Shri Jagdish Prasad Jhabarmal Tibrewala University, Rajasthan. September 2014.

ABSTRACT:

Acne vulgaris is a common human skin disease, characterized by formation of seborrhea, comedone, inflammatory lesions and presence of an aerobic bacteria Staphylococcus epidermidis in the follicular canal and sebum production. Oral and topical antibiotics are the largely prescribed drugs to combat the condition. However bacteria have the ability to develop resistance following repeated or subclinical doses. Hence there is a continual need for newer anti-acne drug molecules for improved drug therapy. Thus the present study aimed to evaluate the hydroalcoholic extract obtained from the bark of Manilkara zapota (L.) Royen, Sapotaceae against S.epidermidis and its potentiation using lemongrass oil. In vitro anti-acne activity using cup plate method and agar dilution method was used to determine the growth inhibition of the organism by the combination of the extract. The results revealed a moderate anti-acne activity of the M. zapota bark extract, however incorporation of the lemongrass oil lead to potentiation of the anti-acne activity by enhancing the growth inhibition against S. epidermidis compared to the standard antibiotics. The study thus concludes that the herbal combination like M. zapota bark extract and lemongrass oil could be used to develop a potential newer anti-acne formulation.
8.3 ORAL PRESENTATION:

Oral Presentation no. 1.


Potentiation of Anti-acne activity of hydroalcoholic extract of Manilkara zapota leaves using Lemongrass oil

Tricia Fernandes, Lonita Lobo, Violet D’Mello, Norma Rebello and Suhasini Bhalerao
St. John’s Institute of Pharmacy and Research, Palghar (E), Bh. Palghar 401404, Maharashtra, India.

1. ABSTRACT: Acne vulgaris is a common bacterial skin disease, characterized by formation of comedones, comedonal inflammatory lesions and presence of an exudate becomes Staphylococcus epidermides and anaerobic bacterial Propionibacterium acnes in the follicular canal and sebaceous production. Oral and topical antibiotics are the largely prescribed drugs to combat the condition. However, bacteria have the ability to develop resistance following repeated or intermittent doses. Hence there is a continuous need for newer anti-acne drug molecules for improved drug therapy. Thus the present study aimed to evaluate the hydroalcoholic extract obtained from the leaves of Manilkara zapota (L.) Roxb. Superb against S. epidermidis and P. acneus and its potentiation using lemongrass oil. In vitro anti-acne activity using cup plate method and area diameters method was used to determine the growth inhibition. The results provided a moderate anti-acne activity of the M. zapota leaf extract, whereas incorporation of the lemongrass oil led to potentiation of the anti-acne activity by enhancing the growth inhibition against S. epidermidis and P. acneus. Hence the study concludes that the herbal combination like hydroalcoholic M. zapota leaves extract and lemongrass oil could be a potential anti-acne drug.

2. INTRODUCTION:

- Common etiology of Acne includes plugged pores with infiltraions of an anaerobic bacterium Propionibacterium acnes and sebaceous duct occlusion.
- S. epidermidis leaves extract has been reported to possess antimicrobial activity against bacteria and fungi due to its tannins, saponins, flavonoids and phenols.
- Essential oils (also called volatile or fragrant oils) are aromatic oils obtained through distillation or steam distillation of the plant material.”

3. METHODOLOGY

- Collection and authentication:
  - Fresh leaves of Manilkara zapota (L.) Roxb., leaves were collected from local gardens of Palghar, S. John’s technical campus, Palghar, Maharashtra, India.
  - Lemongrass oil was procured from Vicer Enterprises, Mumbai.
  - Authenticated by Dr. Basavish M. Patil, Head of Department, Grs. Narsinh Khalsa college of Arts, Science and Commerce, Mumbai, Maharashtra.

- Extraction:
  - Micronization using hydroalcoholic solvent.

- Screening for anti-acne activity:
  1. Cup plate method:
     - Procedure: A hydroalcoholic extract of S. epidermidis and P. acneus was added to 10% DMSO, of the respective agar and poured on pristine plate and well of base diameter were bored. Solution of compound was poured into the wells, the plates were incubated under standard conditions for 24 hours.
  2. Agar diffusion method:
     - Test of extract concentration between (0-1%) was added to broth. Interaction of Agarbroth with compound was measured, lowest concentration for least maximum adherence of growth was observed was recorded.

4. RESULT:

The present study reveals anti-acne activity of hydroalcoholic extract of Manilkara zapota leaves in combination with lemongrass oil. Increased in the diameter of the zones of inhibition against S. epidermidis and P. acneus on addition of lemongrass oil was attributed to the synergistic effect exhibited by lemongrass oil. The MIC value obtained using the extract was higher compared to standard.

5. CONCLUSION

- Significant addition of lemongrass oil to M. zapota leaves extract led to the potentiation of anti-acne activity, the combination of hydroalcoholic extract of M. zapota leaves and lemongrass oil can be used as a herbal drug to treat acne over the synthetic drugs that avoid associated side-effects.

6. REFERENCES

Oral Presentation no. 2.

Oral Presentation no. 3.

Poster Presentation on “In-vitro screening of sunscreen activity of Manilkara zapota leaves extract and its combination with lemon grass oil”. Norma Rebello and Rakesh Mehta at 65th Indian Pharmaceutical Congress, Delhi, December 2013.

Poster No: D-112

IN-VITRO SCREENING OF THE SUNSCREEN ACTIVITY OF Manilkara zapota LEAVES EXTRACT AND ITS COMBINATION WITH LEMON GRASS OIL

Norma Rebello, Rakesh Mehta

St. John Institute of Pharmacy and Research, Palghar (D), Thane, Maharashtra, India.

ABSTRACT:

Solar radiation is a considerable environmental risk factor in the development of non-melanoma skin cancer, sunburn and premature aging of the skin. Traditional medicinal plants can provide a natural solution to this problem. However, research has reported that phototoxic and sunburn reactions may occur when using Manilkara zapota leaves extract and its combination with lemon grass oil. This study was conducted to evaluate the in-vitro sunscreen activity of Manilkara zapota leaves extract and its combination with lemon grass oil.

INTRODUCTION:

Skin damage (especially Ultraviolet radiation, 280 – 400 nm) is caused by harmful, harmful effects, such as long-term exposure to light and the development of skin cancer.

Chemical sunscreens have been reported for photostability and randomized skin reactions. However, the present research work is based on skin sunscreen activity of the extract, specifically hydroxyacetophenone derivatives.

METHODS:

Material and Methods:

Methanol extracts of M. zapota leaves and lemon grass oil were prepared by extraction with methanol. The extracts were diluted with ethanol to obtain concentrations of 0.1, 0.2, 0.4, and 0.6 mg/mL. The sunscreen activity of each extract was evaluated using the OPAW method, which measures the amount of UV radiation absorbed by the extract.

RESULTS:

The results showed that the combination of Manilkara zapota leaves extract and lemon grass oil exhibited a higher sunscreen activity than the individual extracts. The combination showed a higher percentage of UV radiation absorption compared to the individual extracts.

CONCLUSION:

The results obtained prove that the combination of Manilkara zapota leaves and lemon grass oil could serve as a potential natural sunscreen agent, providing effective sunscreen and protection from harmful Ultraviolet radiation. Thus, the chemical sunscreen agents could be replaced with the herbal extract to overcome the documented and measured reactions associated with them.

REFERENCES:
