URL: http://dx.doi.org/10.31703/gpsr.2023(VIII-III).02 DOI: 10.31703/gpsr.2023(VIII-III).02

Citation: Ali, A., Khan, M. S., Abid, H. M. U., & Mukhtar, F. (2023). Determination of Percutaneous Absorption of Polyphenols from Natural Extracts by using Franz Cell. *Global Pharmaceutical Sciences Review, VIII*(III), 18-23. https://doi.org/10.31703/gpsr.2023(VIII-III).02

Cite Us

Vol. VIII, No. III Summer 2023) Pages: 18 – 23 ISSN (Print): 2788-5569 ISSN (Online): 2788-5445

Determination of Percutaneous Absorption of Polyphenols from Natural Extracts by using Franz Cell

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Abstract: *This investigation aimed to explore the bioactive compound potential in medicinal plants such as polyphenols and to test the total phenol content as well as the velocity of skin absorption and antioxidant activities of crude extracts from plants and fruits. albino rats and the Franz diffusion model was used for experimentation. Raphanus sativus proved to have the most substantial phenolic content following Beta vulgaris and Rosa indicat. Analyzed compounds led to skin permeation as Malus domestica had the highest absorbent value among all tested compounds. In addition to that, all extracts were potent DPPH radical scavengers and out of these Daucus carota oil was presented with the best antioxidant ability. The outcome of these experiments advocates for constant research and the growth of highly efficient transdermal delivery systems and aesthetic concoctions with the inherent healing from nature.*

Key Words: Antioxidants, Medicinal Plants, Total Phenolic content, Franz cell, DPPH, Transdermal Drug Delivery System (TDDS)

Introduction

By nature conceived plant species and fruit extracts from medicinal herbs and fruits have attracted considerable attention lately due to their natural origin and diverse health-improving properties they found applications in all relevant fields including skincare, medicine, and nutrition. (Abid et al.). In addition to this, these bioactive compounds are mainly well appreciated for their antioxidant, anti-inflammatory, and antimicrobial characteristics which make them useful ingredients for medicines (synthesis), cosmeceuticals, and functional foods.(Azeem et al., 2023). While Pakistan has a natural endowment of rich biodiversity as well as a tradition of making use of herbal medicines, there is a lack of scientific research that can help understand how these native species affect the skin and are able to pass through it. This lack of knowledge unjustifiably stands in the way of developing nanomaterials for transdermal drug delivery systems, medicine, and skincare products. The research aims to explore the potential of crude extracts from medicinal plants in Pakistan for developing nanomaterials in transdermal drug delivery systems, medicine, and skincare products. This involves determining the total phenolic contents, skin absorption features, and antioxidant capacities of these extracts. By integrating existing research and conducting experimental work, the study seeks to quantify the total phenolic content, assess skin absorption characteristics, and evaluate antioxidant activity. These findings will provide valuable insights into the suitability of these extracts for various applications, potentially leading to the development of novel nanomaterial-based products with beneficial properties for healthcare and skincare. The study will be based on the principle of determining the total phenolic contents of the represented leaves and fruits using the Folin-Ciocalteu assay, which ranks to be

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the best method for phenolic compound estimation. (George et al., 2022). Additionally, it looks to determine the permeation of polyphenols across the skin by employing an in vitro diffusion culture consisting of HaCaT keratinocytes, as well as Franz diffusion cells using excised albino rat skin. (Abid et al., 2022). The other mandate of the study is to estimate the antioxidant activity of the extracts inside the skin by employing the DPPH radical scavenging assay, which is the most common method for antioxidation efficiency calculation.(Usman Abid et al., 2023). Through this research, the target will be availed in order to isolate passive sources of antioxidants and bioactive compounds aimed for transdermal delivery, medicine, food supplements, and cosmetic products. (Abid et al., 2024). The research methodology is structured to achieve the outlined objectives in a systematic manner. It begins with an extensive literature review to establish a foundation for the study and demonstrate its importance to the broader field of natural product research. Following this, the methodology section details the approach for sample collection, preparation, and analysis. Specific experimental protocols are outlined for measuring the total phenolic content of the extracts. Additionally, methods for determining in vitro percutaneous absorption and antioxidant activity are described, emphasizing the use of scientifically validated techniques. This sequential approach ensures that the study is conducted rigorously, with each step designed to contribute to the overall understanding of the potential of these medicinal plant extracts for nanomaterial development. Following the methodology, the findings of the study the researcher summarizes through total phenolic content assessments, skin absorption analysis of polyphenols, and antioxidant activity evaluation. Moreover, cationic liposomes are utilized in both medicine, diet supplements, and cosmetic goods.

Methodology

Sample Collection and Preparation

Various medicinal plants and fruits were selected based on their antioxidant activity. Seeds of Daucus carota, leaves of *Beta vulgaris*, lemon grass stems (*Cymbopogon citratus*), rose petals (*Rosa indica*), and apple pulp (*Malus domestica*) were procured from local markets in Bahawalpur, Pakistan. Pomegranate peels (*Punica granatum*) were obtained by drying until a constant weight was achieved, followed by grinding them into a fine powder. Oil from Daucus carota seeds was extracted using the cold expression method. All plant materials were washed, dried, and powdered before extraction.

Extraction Procedure

Extraction of selected medicinal plants and fruits was carried out using an 80% ethanol-water mixture as the menstruum. Plant material (150 grams) was soaked in 900 ml of ethanol for four days with periodic shaking. After filtration, the filtrates were evaporated using a rotary evaporator at 40°C until one-third of the starting volume was reached. The resulting extracts were stored at 4°C until further analysis.

Determination of Total Phenolic Contents

Total phenolic contents (TPC) were measured using the Folin-Ciocalteu assay. A calibration curve was generated using various concentrations of gallic acid, and the absorbance of plant extract solutions was measured at 760 nm using a microplate reader. TPC was expressed as mg gallic acid equivalents (GAE) per gram of plant extract.

In Vitro Percutaneous Assay

Healthy female albino rat skin was obtained and mounted on Franz diffusion cells. The skin was exposed to extracts applied in the donor compartment, and receptor fluid (potassium dihydrogen phosphate buffer, pH 7.4) was collected over 24 hours. Samples were analyzed for polyphenol content using UV spectrophotometry, and skin permeability was calculated using the permeability equation.

Determination of Antioxidant Activity

Antioxidant activity was assessed using the DPPH radical scavenging assay. DPPH solution was mixed with plant extract samples, and the decrease in absorbance was measured at 517 nm after 30 minutes of incubation. The percentage inhibition of DPPH was calculated and compared to ascorbic acid as a standard antioxidant.

$$(\%)Inhibition = \frac{Blank\ absorbance - Test\ sample\ absorbance}{Blank\ Absorption} \times 100$$

Results and Discussion

Figure 1 depicts the fluctuation in mean absorbance corresponding to variations in Gallic acid concentration. The total phenolic content (TPC) of

chosen medicinal plants and fruits was determined by extrapolating from a Gallic acid calibration curve, utilized as the standard reagent. The results are expressed as mgGAE per gram.

Figure 1

Standard calibration curve of Gallic acid for TPC

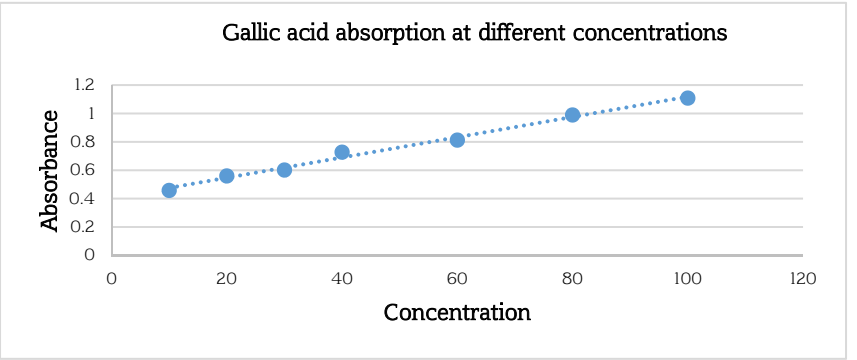
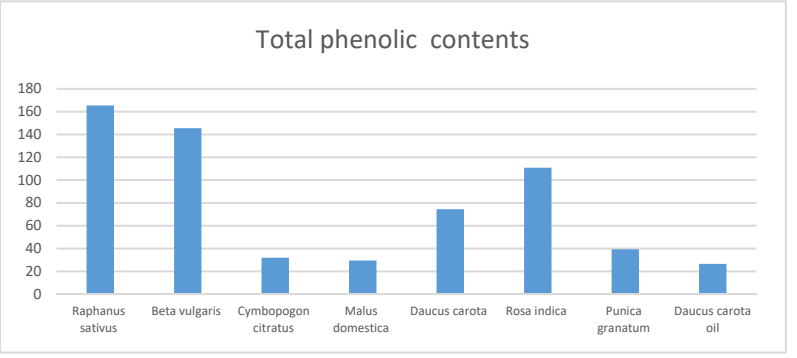


Figure 1

Total phenolic contents in selected plants



Samples were collected from the receptor compartment over a 24-hour period, and their absorbance was measured at 760 nm using a UV-visible spectrophotometer, as illustrated in Figure 3.

Figure 2

Absorption of phenolic contents in the receptor fluid and skin

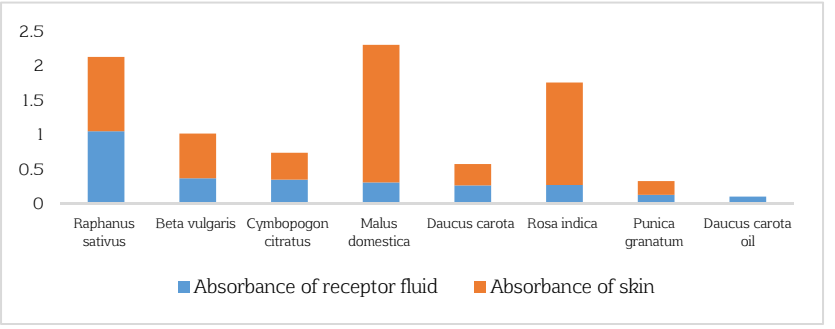


Figure 3

Concentration of Poly Phenols in Samples and skin

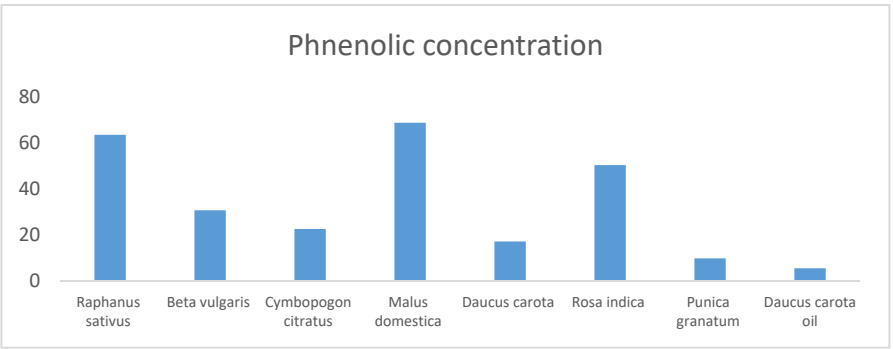
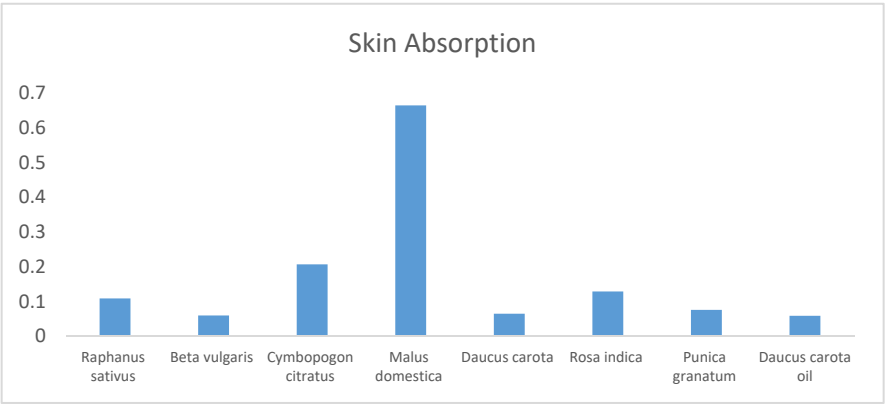


Figure 4

Skin Absorption

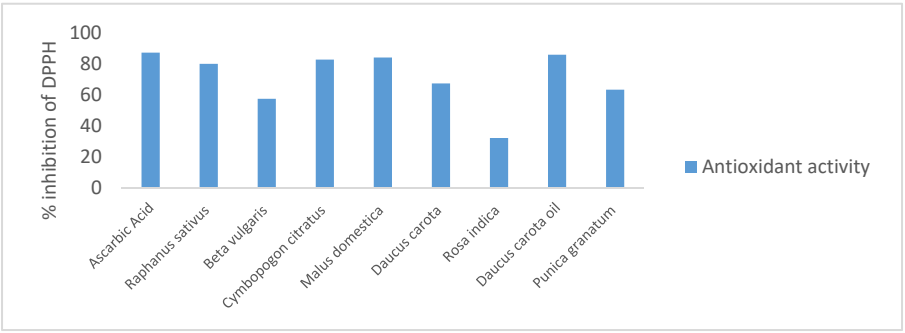


Skin Absorption

Skin permeability was measured by using a permeability equation for each sample, the values of permeability are given in Figure 5.

Figure 5

Antioxidant activity



Anti-oxidant activity by DPPH Method

The antioxidant activity of the compounds and samples produced in the skin assays was assessed by employing ascorbic acid as the standard. Upon the addition of an antioxidant agent to DPPH, the unpaired electron of DPPH becomes paired, causing a color change from purple to yellow. The percentage inhibition of DPPH values compared to the standard is depicted in Figure 6.

The study's findings reveal that certain medicinal plants and fruits from Pakistan are abundant sources of bioactive compounds, especially polyphenols. These compounds have significant potential for applications in diverse fields such as transdermal delivery systems, medicine, and skincare products. The presence of these bioactive compounds suggests that these natural sources could be harnessed to develop innovative products with therapeutic and cosmetic benefits. This highlights the importance of further research and exploration of these natural resources to unlock their full potential for various industries. A combined formed study of total phenolic content demonstrated that Raphanus sativus was the most promising one just slightly ahead of Beta vulgaris and Rosa indica. (Lorenzo et al., 2022). Such findings highlight the marked chemical nature of materials, modulated by conditions such as region and plant tension. Phenolic compounds richness in these extracts proved their utilization as natural antioxidants and skin penetrants. (Akhlaq et al., 2024). In vitro, permeation assays through the skin helped to evaluate the ability of skin-penetrating polyphenol compounds in the extracts and their effect on the biological properties of the skin. The most pronounced skin absorption was detected in the case of Malus domestica which was associated with its lipophilic nature, rich in quercetin, catechin, and other active components. Spectra discrepancies among the extracts warn us of the significance of choosing the right concentration, molecular size, and lipophilicity in the process of delivery to the skin. Antioxidant activity assessments using the DPPH radical scavenging assay were performed across the board for the tested oil extracts. Oil from Daucus carota shows the highest scavenging potential. This extracts' capacity to scavenge free radicals brings to the fore the probability that they have a role to play in the fighting out of damage caused by oxidative stress and the aging of the skin. Still, more research will be key for understanding the specific mechanisms involved and to think of ways to make use of them in practical applications.

Conclusion

The investigated medicinal herbs have shown to be rich sources of bioactive substances, especially polyphenols, which hold great potential for use in transdermal systems, medicine, and skincare products. Their high bioactive content, particularly polyphenols, makes them valuable for various applications. Their diverse chemical compositions, along with their ability to penetrate the skin and exhibit antioxidant properties, have made them subjects of significant research and development efforts across industries. The effectiveness of these medicinal herbs, especially their polyphenols, in transdermal systems, medicine, and skin care products is attributed to their ability to permeate the skin, their varied chemical compositions, and their antioxidant properties. These findings underscore the importance of further exploration and utilization of these natural resources in various industries.

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