

Comparative Study of Maceration, Soxhlet, Percolation Extraction Methods on Bioactive Profile of Poppy Seed Extracts

Uma^{#1}, Hemangi Gehlot^{*2}

[#]Research Intern, Rapture Biotech Jaipur, India

^{*}Junior Research Scientist, Rapture Biotech Jaipur, India

Corresponding author email- rapturebiotechjaipur@gmail.com

Abstract—Papaver somniferum (poppy) seeds are a rich source of bioactive phytochemicals with potential pharmaceutical applications due to their antioxidant and antimicrobial properties. The efficiency of extracting these compounds depends significantly on the extraction method employed, which influences both phytochemical yield and biological activity. The present study aimed to evaluate the effect of different extraction methods on the antioxidant, antibacterial, and antifungal activities of Papaver somniferum seed extracts. Various extraction techniques were compared to identify the most effective method for recovering bioactive compounds. The antioxidant potential of the extracts was assessed using standard free radical scavenging assays, while antibacterial activity was evaluated against selected Gram-positive and Gram-negative bacterial strains. The findings demonstrated that extraction methods significantly affected the recovery of phytochemicals and their corresponding biological activities. Extracts with higher phenolic and flavonoid contents exhibited stronger antioxidant and antimicrobial effects. These results support the traditional medicinal use of poppy seeds and highlight their potential as a natural source of antioxidant and antimicrobial agents. The study provides valuable information for optimizing extraction procedures and may contribute to the development of plant-based pharmaceutical, nutraceutical, and cosmetic formulations aimed at combating oxidative stress and microbial infections.

Keywords—poppy seeds; phytochemicals; antioxidants; antimicrobial activity

I. INTRODUCTION

Medicinal plants have been used for centuries as natural remedies for the prevention and treatment of various diseases. They are rich sources of bioactive phytochemicals such as alkaloids, flavonoids, phenolic compounds, tannins, and saponins, which possess antioxidant, antibacterial, and antifungal properties. The increasing emergence of antimicrobial resistance and the adverse effects associated with synthetic drugs have intensified the search for safe and effective plant-based therapeutic agents [2].

Papaver somniferum L. (poppy) is an important medicinal plant belonging to the family Papaveraceae. Although poppy is widely recognized for its pharmaceutical alkaloids, its seeds are rich in essential fatty acids, proteins, tocopherols, phenolic compounds, and flavonoids that contribute to their nutritional and medicinal value. These phytochemicals are known to exhibit significant antioxidant activity by scavenging free radicals and protecting cells against oxidative damage. In

addition, poppy seed extracts have shown promising antibacterial and antifungal effects against various pathogenic microorganisms [2,3].

The biological activity of plant extracts largely depends on the extraction method used, as different techniques influence the yield and composition of bioactive compounds [4]. Therefore, selecting an appropriate extraction method is essential for obtaining extracts with maximum therapeutic potential. The present study aims to compare different extraction methods for the preparation of poppy seed extracts and to evaluate their antioxidant, antibacterial, and antifungal activities. The findings of this study may provide scientific evidence for the medicinal potential of poppy seeds and support their application in the development of natural pharmaceutical [5].

II. MATERIALS AND METHODS

Collection of Plant Material

Poppy (Papaver somniferum L.) seeds were procured from a local market in Jaipur, Rajasthan,

India. The seeds were cleaned to remove dust and other impurities and stored in airtight containers at room temperature until further analysis [2].

Preparation of Seed Extracts

Three different extraction methods, namely maceration, Soxhlet extraction, and percolation, were employed to prepare methanolic extracts of poppy seeds. For maceration, the seeds were ground into a fine powder, and 1 g of the powdered sample was mixed with 10 mL of methanol. The mixture was placed on a shaker and kept at room temperature for 24 h. After incubation, the extract was filtered through Whatman filter paper, and the filtrate was evaporated to obtain the crude extract [1].

For Soxhlet extraction, the seeds were crushed into a fine powder and preheated in a hot air oven at 105°C for 2 h to remove moisture and facilitate efficient solvent penetration. The dried powder was extracted using methanol in a Soxhlet apparatus until complete extraction was achieved. After extraction, the solvent was removed by simple distillation, and the extract was cooled in a desiccator, weighed, transferred to amber-coloured glass bottles, and stored for subsequent analyses [1].

For percolation, 5 g of powdered poppy seeds were soaked in 150 mL of methanol for 4 h. The soaked material was then packed into a percolator, and extraction was carried out by allowing the solvent to pass slowly through the plant material. Fresh solvent was maintained in contact with the sample for an additional 24 h to ensure complete extraction. The final extract was collected and preserved for phytochemical and biological analyses.

Phytochemical Screening of Seed Extracts

Test for phenolic content: 0.5 mL of the seed extract. The formation of a blue-white colour or precipitate indicated the presence of phenolic compounds in the extract.

Test for Tannins: Tannins were detected by adding a few drops of ferric chloride (FeCl_3) solution to 0.5 mL of the seed extract. The development of a blue-green or dark green colour indicated the presence of tannins in the extract.

Test for Flavonoids: Flavonoids were detected by treating 0.5 mL of the seed extract with a few drops of sodium hydroxide (NaOH). **Test for Phenolic Compounds:** Phenolic compounds were detected by adding 1.5 mL of lead acetate solution to solution,

followed by dilute hydrochloric acid (HCl). The appearance of a yellow colour that disappeared upon the addition of HCl confirmed the presence of flavonoids in the extract.

Test for Terpenoids: Terpenoids were detected by mixing 1 mL of the plant extract with 0.5 mL of chloroform, followed by the addition of 1 mL of concentrated sulfuric acid (H_2SO_4). The formation of a reddish-brown colour at the interface indicated the presence of terpenoids in the extract.

Test for Alkaloids: Alkaloids were detected by adding a few drops of iodine solution to 0.5 mL of the seed extract. The formation of a reddish-brown colour or precipitate confirmed the presence of alkaloids in the extract.

Test for Saponins: Saponins were detected by diluting 0.5 mL of the seed extract with 5 mL of distilled water and shaking the mixture vigorously. The formation of stable, persistent froth or foam indicated the presence of saponins in the extract.

Determination of Antioxidant Activity

The antioxidant potential of the extracts was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Briefly, 0.1 mL of plant extract was mixed with 2 mL of DPPH solution, while methanol and DPPH served as the control and methanol alone served as the blank. The reaction mixtures were incubated in the dark for 30 min, and absorbance was recorded at 517 nm using a UV–Visible spectrophotometer. The percentage radical scavenging activity was calculated using the standard DPPH inhibition formula.

Determination of Total Phenolic Content

Total phenolic content (TPC) was estimated using the Folin–Ciocalteu colorimetric method. Briefly, 0.05 mL of methanolic extract was mixed with distilled water and Folin–Ciocalteu reagent, followed by the addition of 7% sodium carbonate solution. The mixture was incubated in the dark for 30 min at room temperature, and absorbance was measured at 760 nm. Gallic acid was used for the preparation of the standard calibration curve, and the results were expressed as milligrams of gallic acid equivalents (GAE) [1].

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method. The extract was mixed sequentially with distilled water, sodium nitrite, aluminium chloride, and sodium hydroxide. After appropriate incubation, the absorbance was measured at 510 nm using a UV–Visible spectrophotometer. Quercetin was used as the reference standard, and flavonoid content was expressed as quercetin equivalents (QE) [1].

Determination of Total Tannin Content

Total tannin content was determined using the Folin–Ciocalteu method. The extract was reacted with Folin–Ciocalteu reagent and sodium carbonate solution under alkaline conditions to develop a blue-coloured complex. Following incubation, absorbance was measured at 725 nm. Tannic acid was used as the standard, and the results were expressed as tannic acid equivalents (TAE).

Protein Estimation

The total protein content of the extracts was determined by the Lowry method. Standard bovine serum albumin (BSA) solutions were prepared to generate a calibration curve. The extract was treated sequentially with sodium carbonate, copper sulphate, and Folin–Ciocalteu reagent. After incubation, absorbance was measured at 660 nm, and protein concentration was calculated from the BSA standard curve.

Carbohydrate Estimation

Reducing sugar content was estimated using the 3,5-dinitrosalicylic acid (DNSA) method. The extract was mixed with DNSA reagent and distilled water, boiled for 5–10 min, cooled, and the absorbance was measured at 540 nm. D-glucose was used as the standard, and the carbohydrate content was expressed as milligrams of glucose equivalents (GE) per gram of dry extract.

Antibacterial Activity

The antibacterial activity of the methanolic extracts was evaluated by the agar well diffusion method following CLSI guidelines. Nutrient agar plates were prepared, sterilized, and inoculated with standardized cultures of *Escherichia coli* and *Staphylococcus aureus*. Wells of 6 mm diameter were made in the agar, and 20, 40, 60, and 80 μ L of

each extract were added to the respective wells. The plates were incubated at 37°C for 24 h, and antibacterial activity was determined by measuring the diameter of the inhibition zones surrounding each well.

Antifungal Activity

The antifungal activity of the extracts was evaluated against *Malassezia furfur* using the agar well diffusion method. Sterile Sabouraud Dextrose Agar (SDA) plates were inoculated with a standardized fungal suspension, and four wells (6 mm diameter) were prepared in each plate. Different concentrations (20, 40, 60, and 80 μ L) of the extract were introduced into the wells. The plates were incubated at 37°C for 48 h, after which the antifungal activity was assessed by measuring the diameter of the inhibition zones around each well.

III. RESULTS

Phytochemical Screening

The qualitative phytochemical screening of poppy seed extracts obtained by maceration, Soxhlet, and percolation methods is presented in Table 1. The results showed that phenolic compounds were present in the Soxhlet and percolation extracts but absent in the maceration extract. Flavonoids were detected only in the percolation extract, whereas alkaloids were present in both Soxhlet and percolation extracts. Terpenoids were identified in all three extracts, indicating their consistent presence irrespective of the extraction method. Saponins were detected only in the maceration extract, while tannins were absent in all the extracts. These findings demonstrate that the extraction method influenced the qualitative phytochemical profile of poppy seed extracts.

TABLE I. PHYTOCHEMICAL TEST RESULTS

Phytochemical test	Maceration	Soxhlet	Percolation
Phenolic compound	-	+	+
Tannins	-	-	-
Flavonoid	-	-	+
Alkaloids	-	+	+
Terpenoids	+	+	+
Saponins	+	-	-

Determination of total phenolic content

The results of the spectrophotometric determination of the total phenolic content of the extracts obtained

by different extraction methods are presented as follows. The total phenolic content was 0.4524 mg/100 mg for the maceration extract, 3.9786 mg/100 mg for the Soxhlet extract, and 2.86256 mg/100 mg for the percolation extract. Among the three extraction methods, the Soxhlet extract exhibited the highest total phenolic content, followed by the percolation extract, while the maceration extract showed the lowest phenolic content.

Determination of total flavonoid content

The results of the quantitative determination of the extract content obtained by different extraction methods are presented as follows. The maceration extract exhibited the highest content (7.88 mg/100 mg), followed by the percolation extract (1.32412 mg/100 mg), while the Soxhlet extract showed the lowest content (0.33938 mg/100 mg). These results indicate that the maceration method was the most effective for extracting the analyzed constituent among the three extraction techniques.

Determination of total tannins contents

The results of the quantitative determination of the total tannin content of the extracts obtained by different extraction methods are presented as follows. The percolation extract exhibited the highest tannin content (5.9 mg/100 mg), followed by the maceration extract (2.078 mg/100 mg), while the Soxhlet extract showed the lowest content (1.29 mg/100 mg). These findings indicate that the percolation extraction method was the most effective for extracting tannins from the sample.

Determination of total protein content

The results of the quantitative determination of the protein content of the extracts obtained by different extraction methods are presented as follows. The maceration extract exhibited the highest protein content (1.6923 mg/100 mg), followed by the Soxhlet extract (0.99215 mg/100 mg), while the percolation extract showed the lowest protein content (0.159 mg/100 mg). These findings indicate that the maceration extraction method was the most effective for extracting proteins from the sample.

Determination of total carbohydrates

The results of the quantitative determination of the total carbohydrate content of the extracts obtained

by different extraction methods are presented as follows. The maceration extract exhibited the highest carbohydrate content (6.1818 mg/100 mg), followed by the percolation extract (2.33615 mg/100 mg), while the Soxhlet extract showed the lowest content (1.23155 mg/100 mg). These findings indicate that the maceration extraction method was the most effective for extracting carbohydrates from the sample.

Antioxidant Activity

The antioxidant activity of the extracts obtained through different extraction methods showed *significant* variation. Among the three techniques evaluated, percolation yielded the highest antioxidant activity (80.69%), followed by Soxhlet extraction (48.89%), while maceration exhibited the lowest activity (25.22%).

Antimicrobial Activity

The antimicrobial activity of poppy seed extracts was evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus sp.*, and *Candida sp.* using the agar well diffusion method. The maceration extract showed the highest antimicrobial activity, producing the largest zones of inhibition against all tested microorganisms. The percolation extract exhibited moderate activity against *E. coli* but showed no inhibition against the other microorganisms. In contrast, the Soxhlet extract did not exhibit any significant antimicrobial activity. These findings indicate that the maceration method was the most effective for extracting antimicrobial compounds from poppy seeds.

TABLE II. ZONE OF INHIBITION OF *Papaver somniferum* SEED EXTRACTS AGAINST *E. coli*

Extraction method	Zone of inhibition (mm)
Maceration	9.5
Soxhlet	0
Percolation	8.305

TABLE III. ZONE OF INHIBITION OF *Papaver somniferum* SEED EXTRACTS AGAINST *S. aureus*

Extraction method	Zone of inhibition (mm)
Maceration	10.24
Soxhlet	0
Percolation	0

TABLE IV. ZONE OF INHIBITION OF *Papaver somniferum* SEED EXTRACTS AGAINST *Aspergillus niger*

Extraction method	Zone of inhibition (mm)
Maceration	0
Soxhlet	0
Percolation	0

TABLE V. ZONE OF INHIBITION OF *Papaver somniferum* SEED EXTRACTS AGAINST *Candida albicans*

Extraction method	Zone of inhibition (mm)
Maceration	10.735
Soxhlet	10.155
Percolation	0

IV. DISCUSSION

The present study demonstrated that the maceration method was the most effective extraction technique for poppy seeds, yielding higher amounts of phytochemicals and better biological activity than the Soxhlet and percolation methods. The enhanced extraction efficiency may be attributed to prolonged solvent-sample contact, which facilitated the release of bioactive compounds. The superior antioxidant and antimicrobial activities of the maceration extract are likely associated with its higher phytochemical content. These findings suggest that maceration is a simple, cost-effective, and efficient method for extracting bioactive constituents from poppy seeds for phytochemical and pharmacological studies.

V. CONCLUSION

The present study demonstrated that the maceration method was the most effective extraction technique for poppy seeds, yielding higher phytochemical content and superior antioxidant and antimicrobial activities compared to the other extraction methods. The findings indicate that methanolic maceration is a simple, cost-effective, and efficient approach for extracting bioactive compounds. Therefore, poppy seeds may serve as a promising natural source of therapeutic agents for future pharmaceutical and nutraceutical applications.

VI. REFERENCES

[1] Kothari, V. (2012). Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds.
[2] Edewor, T. I., Akpor, O. B., & Owa, S. O. (2016). Determination of antibacterial activity, total phenolic, flavonoid and saponin contents in leaves

of *Anogeissus leiocarpus* (DC.) Guill and Perr. J Coast Life Med, 4(4), 310-4.
[3] da Silva Oliveira, F. G., de Lima-Saraiva, S. R. G., Oliveira, A. P., Rabêlo, S. V., Rolim, L. A., & da Silva Almeida, J. R. G. (2016). Influence of the extractive method on the recovery of phenolic compounds in different parts of *Hymenaea martiana* hayne. Pharmacognosy Research, 8(4), 270.
[4] Patel, N. I. V. E. D. I. T. A., Patel, P., Patel, D. H. A. R. A., Desai, S. H. A. R. A. V., & Meshram, D. (2014). Phytochemical analysis and antibacterial activity of *Moringa oleifera*. International Journal of Medicine and Pharmaceutical Sciences, 4(2), 27-34.
[5] Thapa, N., Thapa, P., Bhandari, J., Niraula, P., Shrestha, N., & Shrestha, B. G. (2016). Study of phytochemical, antioxidant and antimicrobial activity of *Artocarpus heterophyllus*. Nepal Journal of Biotechnology, 4(1), 47-53.
[6] Lachenmeier, D. W., Sproll, C., & Musshoff, F. (2010). Poppy seed foods and opiate drug testing-where are we today? Therapeutic Drug Monitoring, 32(1), 11-18.
[7] Chaudhary, S. S., Aslam, M., & Siddiqui, A. (2019). Tukhm khashkhaash (poppy seeds): A unani drug of multitudinous potential. J. Drug Deliv. Ther, 9, 528-533.
[8] Zahid, Z., Khan, S., Nadeem, F., & Azeem, M. W. (2015). The review of power of Poppy: Harnessing benefits of nature's most dangerous Plant. International Scientific Organization, 8, 56-64.
[9] Bhoir, V. In-vitro screening of inhibitory activity of Poppy seeds (*Papaver somniferum* Linn.) extract against *Malassezia furfur*.
[10] Chaudhry, N. M. A., & Tariq, P. (2008). In vitro antibacterial activities of kalonji, cumin and poppy seed. Pakistan Journal of Botany, 40(1), 461.
[11] Satranský, M., Fraňková, A., Kuchtová, P., Pazderů, K., & Capouchová, I. (2021). Oil content and fatty acid profile of selected poppy (*Papaver somniferum* L.) landraces and modern cultivars. Plant, Soil & Environment, 67(10).
[12] Kusmenoglu, S., Akay, Z., & Sener, B. (2002). Fatty acid composition in the seed oils of *Papaver somniferum* from different provinces. FABAD J. of Pharm. Sci, 27, 13-18.
[13] Khan, M. R., Khawar, Z., & Latif, M. (2012). Biochemical Investigation of Oil of *Papaver somniferum*. Asian Journal of Chemistry, 24(10), 4476.

- [14] Kumaravel, S., & Alagusundaram, K. (2014). Antimicrobial activity and phytochemical analysis of selected Indian spices. *Journal of Pure and Applied Microbiology*, 8(5), 4131-4136.
- [15] Meena, Y., Kant, L., Pandey, A., & Suradkar, U. S. (2024). Effect of Poppy (*Papaver somniferum*) seed extract on the Physico-chemical, microbial and sensory Characteristics of Chevon patties.
- [16] Sproll, C., Perz, R. C., Buschmann, R., & Lachenmeier, D. W. (2007). Guidelines for reduction of morphine in poppy seed intended for food purposes. *European Food Research and Technology*, 226(1), 307-310.
- [17] Singh, R., Kapoor, D., Sharma, R., & Mahajan, P. (2023). A review on Poppy seeds and its organoleptic behaviour. *Int J Scientific Res Sci Technol*, 10(4), 30-34.
- [18] Zahid, Z., Khan, S., Nadeem, F., & Azeem, M. W. (2015). The review of power of Poppy: Harnessing benefits of nature's most dangerous Plant. *International Scientific Organization*, 8, 56-64.
- [19] Mishra, S., & Pathak, V. (2021). PHYTOCHEMICAL STUDY AND ANTIMICROBIAL ACTIVITY OF KHUS-KHUS (*PAPAVR SOMNIFERUM*) SEEDS.
- [20] Chan, K. W., Iqbal, S., Khong, N. M., & Babji, A. S. (2011). Preparation of deodorized antioxidant rich extracts from 15 selected spices through optimized aqueous extraction. *J. Med. Plants Res*, 5(25), 6067-6075.
- [21] Kaynarca, G. B., Karabulut, Ş. Y., Güleç, H. A., & Kamer, D. D. A. (2025). Characterization of Red Poppy (*Papaver rhoeas* L.) Extract: An Alternative Food Colorant. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 28(4), 1103-1121.
- [22] Suissa, J., & Barton, K. E. (2018). Intraspecific and interspecific variation in prickly poppy resistance to non-native generalist caterpillars. *Botanical Sciences*, 96(2), 168-179.
- [23] Yilmaz, E., & Emir, D. D. (2017). Compositional and functional characterisation of poppy seed (*Papaver somniferum* L.) press cake meals. *Quality Assurance and Safety of Crops & Foods*, 9(2), 141-151.
- [24] Gracelin, D. H. S., Britto, A., & Kumar, B. J. R. P. (2013). Qualitative and quantitative analysis of phytochemicals in five *Pteris* species. *Int J Pharm Pharm Sci*, 5(1), 105-107.
- [25] Das, K., Kalita, P. P., Sarma, M. P., Talukdar, N., & Kakoti, P. (2014). Extraction, estimation and comparison of proteins and carbohydrates from different parts of *Costus speciosus* and a brief study on its phytochemicals content. *International Journal of Basic and Applied Biology*, 2(2), 81-85.
- [26] Martens, D. A., & Loeffelmann, K. L. (2002). Improved accounting of carbohydrate carbon from plants and soils. *Soil Biology and Biochemistry*, 34(10), 1393-1399.