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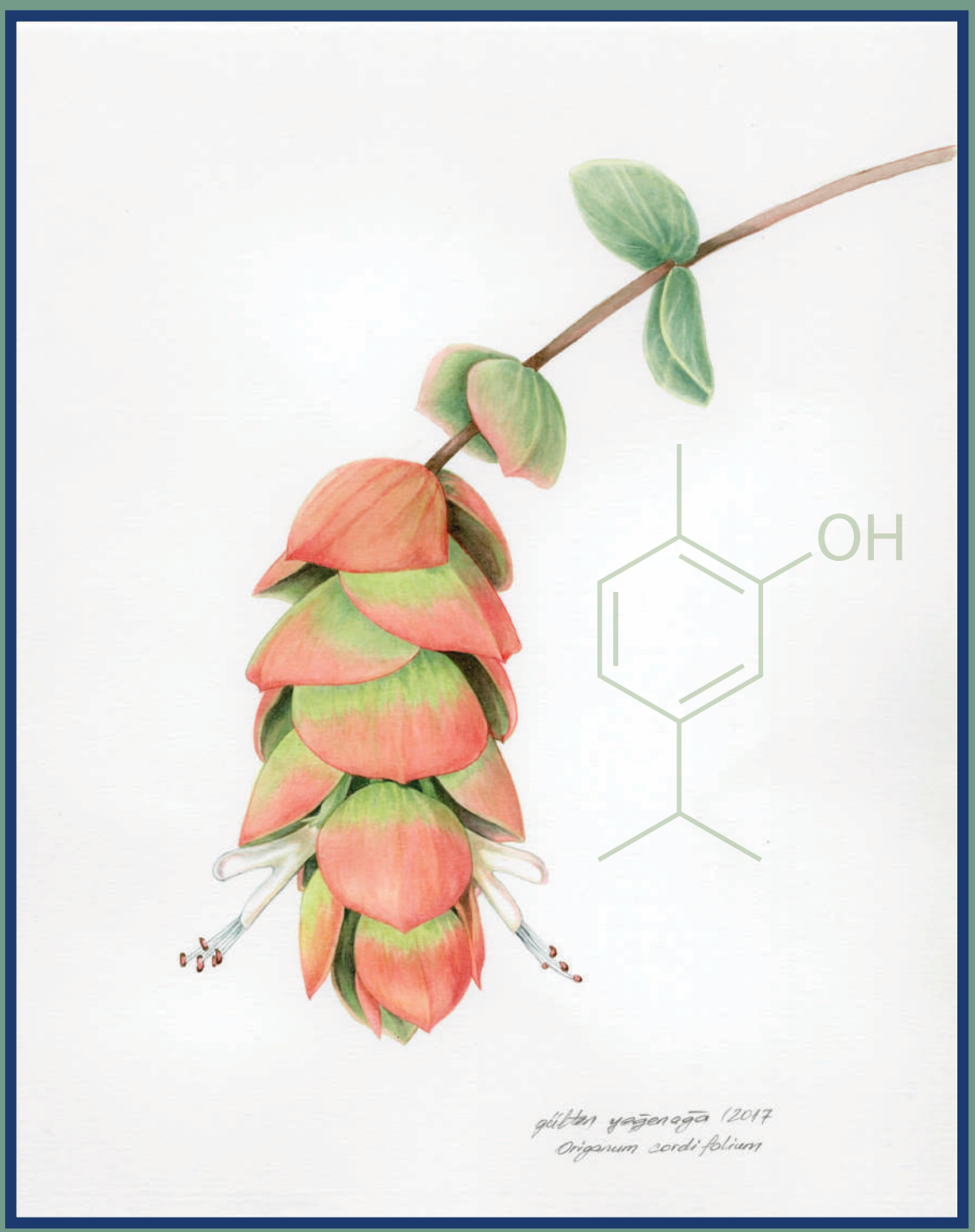

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Origanum cordifolium* **in cover picture was illustrated by Gülten Yeğenağa

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MESSAGE FROM THE EDITOR IN CHIEF

Dear Colleagues,

It is a pleasure to introduce the 2nd issue of EMU Journal of Pharmaceutical Sciences Volume 8, 2025. I would like to congratulate all participants, editorial board, and the journal secretariat for their dedicated work.

As a member of 'DergiPark' Akademik, an establishment under the Scientific and Technological Research Council of Türkiye (TÜBİTAK), EMU Journal of Pharmaceutical Sciences continues its journey with a transparent peer-review and publication process of scientific studies in diverse fields related to pharmaceutical sciences. The journal will continue to serve and promote global dissemination of pharmaceutical research, providing a platform for scientists worldwide. It is important to remind that the journal is free of submission or acceptance fee.

In the near future, we are planning to launch the 3rd issue of the year.

Looking forward to your scientific contributions,

Best wishes,

Prof. Dr. H. Ozan Gülcan

Dean of Faculty of Pharmacy
Editor in Chief
Eastern Mediterranean University
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GUIDE FOR AUTHORS

EMU Journal of Pharmaceutical Sciences (EMUJPharmSci) covers the research on all aspects of Pharmacy presented as original articles, short reports and reviews.

EMU Journal of Pharmaceutical Sciences is published three times (March, July, November) each year. It is an open access and peer-reviewed journal.

- Contributions to EMU Journal of Pharmaceutical Sciences must be in English.
- All manuscripts are subject to editorial review.
- The manuscripts should not be previously published or accepted for publication and should not be submitted or under simultaneous consideration for publication elsewhere.
- The manuscripts are published in the order of final acceptance after review and revision.
- If the manuscript is returned to authors for revision and the revised manuscript is not received by the editor within 2 months it will be treated as a new article.
- If the manuscript is accepted and the proof is returned to the authors, corrected proofs should be sent to the editor within 5 days.

Original articles: These are limited to 15 typewritten pages in addition to supplementary materials (schemes, tables, figures, etc.).

Short papers: Short papers are limited to 5 typewritten pages and maximum of 2 supplementary materials (schemes, tables, figures).

Reviews: They are limited to 20 pages in addition to supplementary materials (schemes, tables, figures, etc.).

- The original manuscript must be arranged as follows: Title page (including the title, authors and correspondence address), abstract, key words, introduction, materials and methods, results and discussion, acknowledgements and references.
- The reviews must be arranged as follows: Title page (including the title, authors and correspondence address), abstract, introduction, discussion, acknowledgements and references.

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1. General Format

a) All manuscripts can only be submitted electronically via DergiPark.
b) Manuscripts should be 1.5-lines spaced and justified.
c) Use 2.5 cm margins and Times New Roman font on A4 paper.
d) Number all pages, starting with the title page.
e) Spell out all acronyms in full at first use.
f) Make sub-headings if necessary.
g) Follow internationally accepted rules and conventions: use the international system of units (SI).

2. Before main text

A. Title page

a) The first page of the manuscript is a title page containing the following information:
b) The manuscript's full title (*Font: Times New Roman Font Size: 13*). The title must be concise and informative.
c) All authors' full names (*Font: Times New Roman Font Size: 11)*.
d) The affiliation of the author(s) should be linked by superscript numbers, and listed beneath the title.
e) All authors' ORCID ID number (*Font: Times New Roman Font Size: 11)*.
f) Corresponding author (*Font: Times New Roman Font Size: 10)*. E-mail, telephone and fax number (with country and area code) of the corresponding author should be provided.
g) Ethical approval must be provided for studies involving human or animal participants.

B. Abstract

a) The abstract appears on its own page.
b) The abstract should be written in Times New Roman and font size 11.
c) The maximum length of the abstract is 200 words.
d) The abstract should contain the objectives, methods, results and conclusions.
e) 3- 6 key words must be provided in alphabetical order (Font: Times New Roman Font Size: 10). Separate keywords with commas.

3. Main text

A. Introduction

(Font: Times New Roman Font Size: 12)
State the objectives of the work and provide a brief background of the literature related to the topic. The novelty and the aim of the study should be clearly stated.

B. Materials and Methods

(Font: Times New Roman Font Size: 12)
a) Give a brief and clear description of the materials and methods used. Subtitles can be given as appropriate.
b) For plant materials, herbarium name (or acronym), number, name and surname of the person who identified the plant materials should be indicated in this part of the manuscript.
c) Statistical analysis must be provided when necessary

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C. Results and Discussion

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A combined Results and Discussion section is often appropriate. Results should be concise.

Discussion should explore the significance of the results of the work.

Discussion should not repeat the results.

The main conclusions of the study should be presented.

D. Acknowledgement

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Supporting institutions or individuals should be briefly acknowledged just before the reference list.

E. References

i. Citation in text

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- Please ensure that every reference cited in the text is also present in the reference list (and vice versa).
- Unpublished results and personal communications are not recommended in the reference list.
- References in the text should be cited as: the author(s) surname and the publication date**.**

Examples:

(Sahin, 2000) – one author

(Sahin and Kosar, 2000) – Two authors

(Sahin et al., 2000) – more than two authors

(Celik and Ozhatay 2000 a, b) – More than one paper in the same year by the same author (s)

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- List the references in alphabetical order under section of "references".
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- Volume numbers should be indicated in bold letters.

iii. Examples

Reference to a journal publication:

Ozhatay N, Kultur S, Gurdal B (2017). Check-list of additional taxa to the supplement flora of Türkiye VIII. *Istanbul J Pharm* **47**(1): 31-46.

Ozhatay N, Kultur S, Gurdal B, Ilktac M, Ogmen S, et al. (2019). Check-list of additional taxa to the supplement flora of Türkiye X. *Istanbul J Pharm* **57**(2): 35-46.

Reference to a book:

Strunk W Jr, White EB (1979). The Elements of Style. 3rd ed. New York, NY: Macmillan.

Reference to a chapter in an edited book:

Bonati A (1988). Industry and conservation of medicinal plants. In Akerele O, Heywood V, Synge H (eds). The Conservation Medicinal Plants p.141-148 Cambridge University Press UK.

Electronic resources:
World Nuclear Association (WNA) (2014). Radioisotopes in Medicine, http://www.world-nuclear.org/info/ Accessed 13.10.2014.

4. After main text

Figures / Tables captions

- Use figures and tables for information better presented visually.
- All the figures and tables must be referred to in the main body of the text.
- Tables and Figures should be numbered consequently in the order of appearance within the text, referred as "Table 1" and "Figure 1".
- Descriptive titles should be given at the top of the tables and at the bottom of the figures.
- Figures should be prepared with the highest resolution and should be provided as a separate page following references.

Submission checklist

Check the following submission list before submit your manuscript:

- Full E-mail address, full postal address, telephone and fax number of the corresponding author.
- All necessary files have been uploaded.
- References are in the correct format for this journal.
- All references mentioned in the Reference list are cited in the text.
- All figure captions.
- All tables (including title, description, footnotes).

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Aromatherapeutic Prescription Patterns and Their Evaluation in the Case of the Beyazıt Halk Pharmacy in Istanbul (Türkiye)

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Abstract

This study aims to evaluate the scientific basis of aromatherapeutic applications by analyzing the botanical contents, frequency of use and therapeutic purposes of aromatherapy prescriptions used in the field of health in Türkiye.

The prescriptions obtained from the archives of Istanbul Beyazıt Halk Pharmacy were examined and evaluated in terms of the herbal species, families, usage values (UV), functional consensus index (FIC) and chemical components included in the prescriptions.

According to the data obtained, the most frequently used plants in prescriptions are *Lavandula angustifolia, Matricaria chamomilla*, *Salvia rosmarinus* and *Eucalyptus globulus*; these plants are seen to have high UV values. FIC analysis revealed a high consensus in some therapeutic categories, especially respiratory disorders (FIC = 0.87). In addition, it was determined that the majority of the analyzed plants contain volatile components with scientifically proven therapeutic effects.

These findings show that aromatherapy is increasingly being adopted in both traditional and modern medical approaches in Türkiye and that its prescription use is beginning to gain a certain systematic structure. However, in order to make aromatherapy applications more effective and safer, clinical guidelines need to be established and scientifically based standardization processes need to be developed.

Keywords

Aromatherapy, functional consensus index, medicinal plants, prescription analysis, Türkiye, use value.

Article History

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INTRODUCTION

The first humans had the opportunity to experience nature very closely by living in a natural environment. The sense of smell, which is ten thousand times more sensitive than the sense of taste, was extremely important for them to sustain life, to estimate the location of existing dangerous animals and enemies, and to determine the location of food. Paintings from the pre-Christian period on the walls of the Lascaux cave in France show medicinal plants. The first humans associated bad smells with God's disapproval, discomfort or disease. According to them, a healthy person has a clean and fresh smell. Plants and spices protect food, help digestion and increase taste. Again, the first humans, by chance, noticed the pleasant and healing smoke that some plants emitted when they were dropped into fire and began to use resin and dried herbs in purification ceremonies. Over time, plants and aromatics became one of the few important things to be obtained, protected and continued (Miller and Miller, 2001; Hudson, 1998). Papyrus records dating back to 4500 BC show that balsam, perfumed oils, scented tree barks and resins were used and aromatic mixtures were produced. Believing that the physical body was important after life, the Egyptians developed the art of mummification by discovering aromatic and volatile oils with protective effects. The Mesopotamian, Indian and Chinese civilizations also exchanged information on herbal and aromatic products and how to use them. In India, Ayurveda, plants and aromatics are considered an important part of the philosophy of healing. The Greeks and Romans acquired the science of aromatherapy, the use of perfumes, scented oils and the understanding of herbal treatment from the Egyptians and established a connection with Indian culture (Brahms, 2004; Hudson, 1998).

Doctor Rene Maurice Gattefosse is considered the modern father of aromatherapy. When he realized that his hand, which was severely burned in a laboratory explosion, could be treated with lavender oil and the pain was reduced, he devoted the rest of his life to scientific research on the therapeutic properties of essential oils. He first coined the term ‘aromatherapy’ and published a book of the same name in 1937. French medical doctor Jean Walnet was greatly influenced by Gattefosse's work and successfully healed war wounds with antiseptic essential oil solutions during World War II. Later, the American Aromatherapy Association was founded in England under the leadership of Mallory, who was a student of Dr. Valnet. The use of aromatherapy began to increase

in the United States as the limitations of modern medicine and the importance of self-care were understood and the potent effects of essential oils were recognized (Brahms, 2004; Hudson, 1998).

Aromatherapy is the controlled use of essential oils obtained from plants in the treatment of physical and psychological disorders. Studies on aromatherapy in Türkiye have focused especially on nursing (Kurtgoz and Kiziltepe, 2022). Aromatherapy applications are used in the management of various health problems in Türkiye. It is stated that it can positively affect a person's mood and physical health in cases such as stress, sleep disorders, and menstrual cramps. It is also used in the treatment of disorders such as headache, muscle and joint pain, skin and hair problems (Kurtsan, 2021). Studies and applications on aromatherapy in Türkiye are increasing and it is considered as a complementary method in the management of various health problems. In this study, the use of aromatherapy for health purposes was evaluated with a statistical approach through aromatherapy prescriptions written by physicians.

Established in 1950, Beyazıt Halk Pharmacy is known as one of the oldest and most established pharmacies in Istanbul (Figure 1). Serving in the same location since its establishment, the pharmacy provides health services in the region with its long-standing experience and professional staff. The pharmacy's activities, which have continued for more than half a century, are supported by customer trust and a sustainable service approach. Beyazıt Halk Eczanesi is located in Beyazıt Square, which is considered the historical center of Istanbul, in a location adjacent to important cultural and historical structures. Its proximity to the main gate of Istanbul University, Beyazıt Mosque and the Grand Bazaar allows the pharmacy to be frequently preferred not only by locals but also by tourists. This location increases the diversity of the customer base that the pharmacy serves, allowing it to become a structure that appeals to both local and foreign visitors. The Beyazıt Halk Pharmacy, which contributed to this study, is considered a reliable reference point in the region for health services due to its long history. In addition to prescription drugs, the pharmacy also offers herbal and aromatherapeutic products, personal care products and medical supplies. The pharmacy's deep-rooted history, experienced staff and wide range of products allow it to provide reliable consultancy for health services.

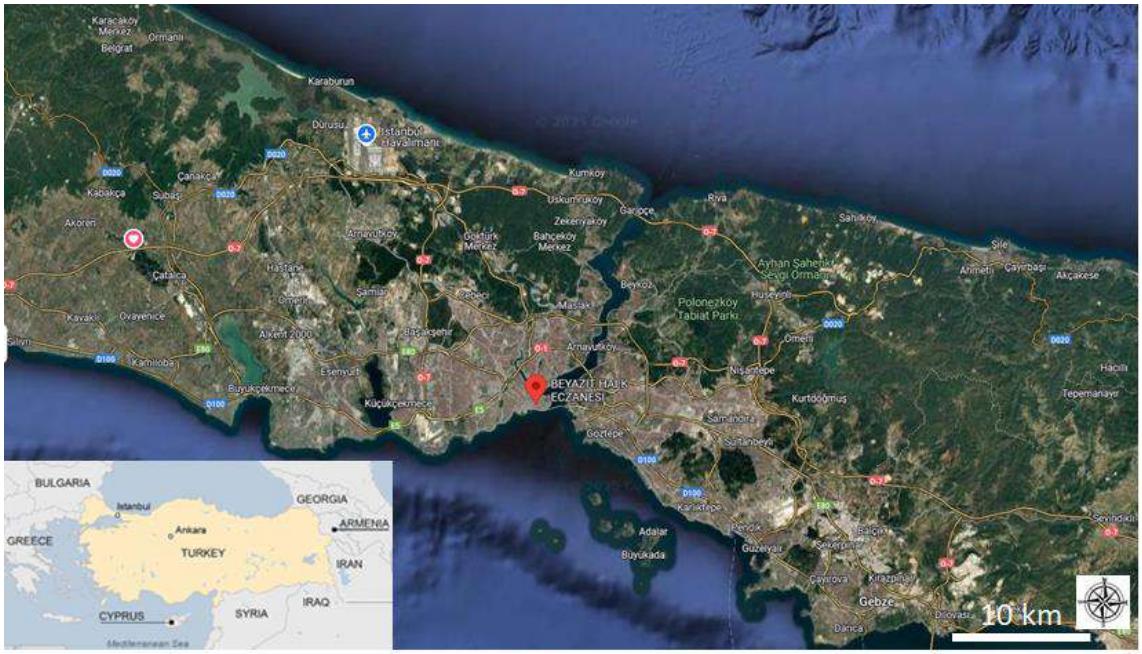

Figure 1: Location of Beyazıt Halk Pharmacy in Istanbul.

MATERIALS AND METHODS

During the first six months of 2020, 47 aromatherapeutic prescriptions received by Beyazıt Halk Pharmacy were recorded for review (Figure 2). The prescriptions were prepared by the first author himself. The essential oils used in the preparation of the prescriptions subject to this study were grouped into 3 classes:

I. 100% pure: not mixed with other essential oils

II. 100% natural: natural qualities not impaired by synthetic molecules

III. 100% holistic: not subjected to any application that would eliminate the molecules

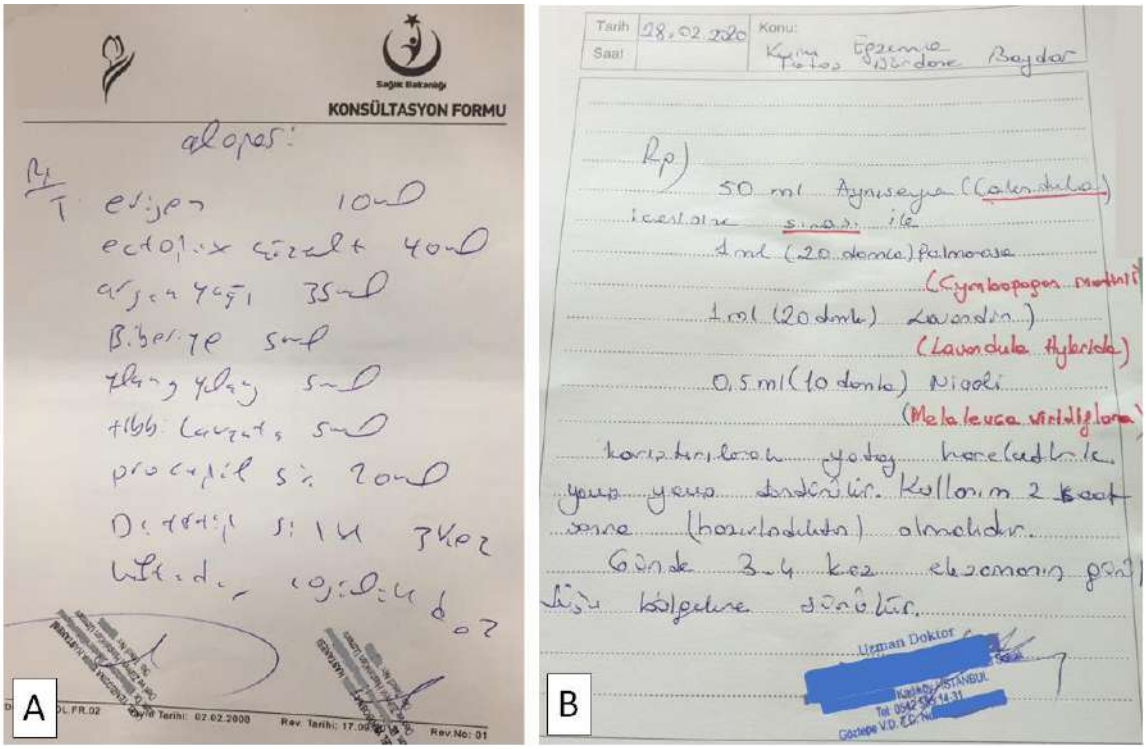

Figure 2: A prescription written for the treatment of alopecia (A), and eczema (B).

The oils used in the prescriptions are botanically and biochemically defined essential oils and are organic certified. The EOBBD (Essential Oil Botanically and Biochemically Defined) Certificate is a certificate that guarantees the quality, purity and chemical structure of essential oils. It was developed specifically to ensure the therapeutic effect of essential oils used in aromatherapy. This certificate is usually issued by international independent certification bodies such as Ecocert. Ecocert is a worldwide known and reliable certification body for organic agriculture and natural cosmetic products. Before preparing the recipes, essential oils are subjected to organoleptic analysis in order to evaluate their physical and chemical properties. These analyses include the evaluation of sensory properties of oils such as odor, color, taste and viscosity by experts. Organoleptic evaluation is considered a basic method for determining the originality, purity and quality of essential oils and is supported by chemical analysis. This process aims to ensure the therapeutic efficacy and reliability of oils (Figure 3).

Figure 3: Organoleptic control of oils before the preparation of recipes.

Also, Informant consensus factors (ICF or FIC) and the use-value UV formulas have been updated based on this study. ICF were calculated as [FIC=(Nur-Nt)/(Nur-1)] (Trotter and Logan, 1986). Total usage report ($Nur_{recipes}$): Number of all herb uses in recipes belonging to that category (if the same herb is used in more than one recipe, each one is counted). Total different species ($Nt_{recipes}$): The number of different plant species used in recipes belonging to that category. The UV value (Abu-Irmaileh and Afifi, 2003) was calculated both among the total plants used and among the plants in the recipes prepared for each ailment. UV=U/N, UV is the use-value of a species; U= the number of citations for every species in this recipes; N= the number of recipes. $UV_{ailment}$ =U/N, $UV_{ailment}$ is the use-value of a species for each ailment; $U_{ailment}$= the number of citations for every species in this recipes for each ailment; $N_{ailment}$= the number of recipes for each ailment.

RESULTS

As a result, 47 recipes containing 59 species belonging to 29 families are listed in Table 1. The scientific names of the species, their commercial names, families and the values used in the recipes are also listed in Table 2. It is clearly seen that aromatherapy prescriptions are preferred for skin diseases (FIC=0.62) (Figure 4). After this disease, aromatherapy is used for upper respiratory tract problems (FIC= 0.33), immune system problems (FIC= 0.18), mental (FIC= 0.16), and women's health disorders (FIC= 0.12). The taxa with the highest UV value are *Cananga odorata* (Lam.) Hook.f. & Thomson (0.22), *Lavandula angustifolia* Mill. (0.32), *Salvia rosmarinus* Spenn. (0.17), *Sideroxylon spinosum* L. (0.16), *Helichrysum* Mill. (0.13), and *Melaleuca alternifolia* (Maiden & Betche) Cheel (0.13).

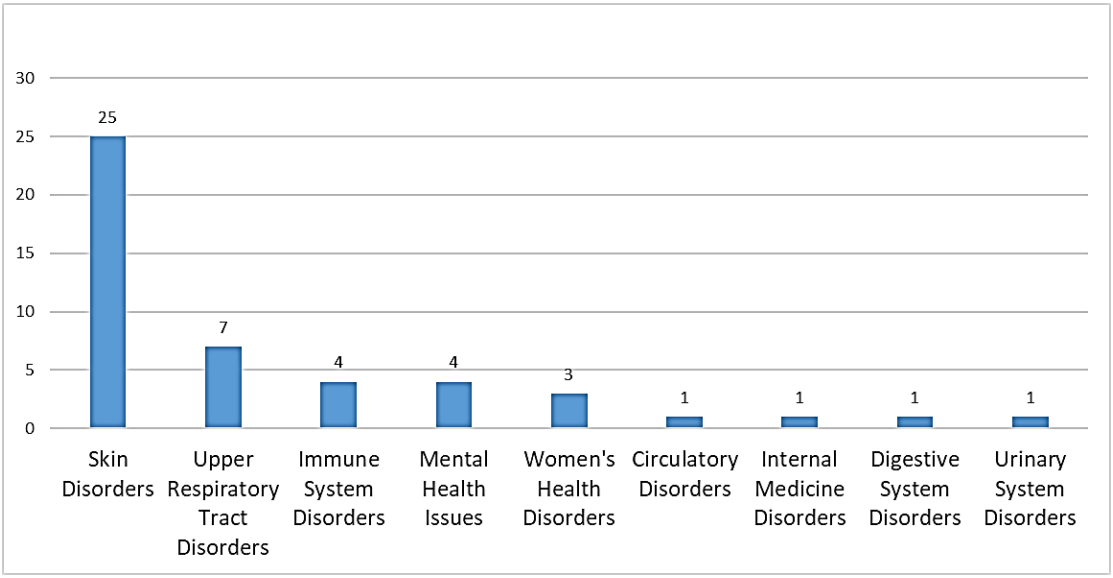

Figure 4: Frequency of use of aromatherapeutic prescriptions according to diseases.

The frequency of use of the first three plants most commonly used for each disease diagnosed in prescriptions was also calculated. According to these results, the species with the highest frequency of use in skin disorders are lavender ($UV_{skin\ disorders}$=0.12), chamamille ($UV_{skin\ disorders}$=0.08), and argan ($UV_{skin\ disorders}$=0.07). The plant with the highest usage value in prescriptions for upper respiratory tract problems is eucalyptus ($UV_{Upper\ respiratory}$=0.14), while peppermint, ginger and juniper also have the same usage frequency value and are the plants ranked 2nd ($UV_{Upper\ respiratory}$=0.09). The three most commonly used herbs in immune system-supporting prescriptions are jojoba, eucalyptus and peppermint, and the frequency of use value for all of them is same ($UV_{Immune\ System}$=0.09). In the prescriptions prepared for mental problems, two plants stand out. These are lavender

and rosemary, their frequency of use is equal ($UV_{Mental\ Health}$=0.1).

In the recipes prepared for women's health problems, 4 plants that are not used very often in other diseases are at the forefront. These are ylang-ylang, myrtle, clary sage, and sweet almond oil, their frequency of use is equal ($UV_{Women's\ Health}$=0.11). In the recipes prepared for women's health problems, cod fish oil, which is an animal product, is also used. Instead, vegetable oils such as Flaxseed oil, Chia seed oil, Hemp oil, which are rich in omega-3, can be used. The genera represented with the most species in the prepared recipes are *Citrus*, *Melaleuca*, and *Salvia* (Figure 5). The families represented by the most genera in the prepared recipes are Lamiaceae, Myrtaceae, Rosaceae and Lauraceae (Figure 6).

Table 1: Contents and application methods of aromatherapy prescriptions according to diseases.

Diagnosed Health Issue	Prescribed Herbal Oils	Preparation and Usage
Acne (adolescence)	- 50 ml lavender hydrosol (1.5%) - 2 drops manuka - 3 drops tea tree oil - 2 drops medical lavender - 3 drops blue chamomile	Applied 4-5 times a day.
Acne & Sunspots	- 50 ml argan oil - 20 drops Helichrysum - 20 drops tea tree oil	Applied once a day.
Alopecia Areata	- 10 ml Vitamin E ampoule - Terbinafine-containing preparation - 40 ml lotion - 3 ml argan oil - 5 ml rosemary - 5 ml ylang-ylang - 5 ml medical lavender - 20 ml Procapil 5%	Applied 3 times a day for 10 days as a powder.
	- 10 ml jojoba oil - 10 ml argan oil - 2 drops rosemary - 3 drops lavender - 2 drops oregano - 3 drops cedarwood	Massaged into the scalp for at least 4 minutes. After massaging, wash hands. Cover the hair with a shower cap and leave for 1-2 hours. Hair can be washed after.
	- 50 ml argan oil - 10 drops rosemary oil - 10 drops Helichrysum - 10 drops lavender	Applied morning and evening.
	- 30 drops argan oil - 30 drops castor oil - 30 drops lavender - 25 drops thyme - 5 drops rosemary	Applied morning and evening.
Anxiety	- 120 ml carrier oil - 6 drops vetiver - 6 drops medical lavender	Applied using a diffuser.
Aphthous Ulcer (AFT)	- 50 ml argan oil or 50 ml rose hydrosol - 5 drops tea tree oil - 2 drops peppermint - 1 drop blue chamomile - 2 drops medical lavender or fennel	Applied as a spray.

Atopic Dermatitis - Dry Skin	- 5 drops chamomile - 3 drops medical lavender - 1 drop rose oil - 1 drop patchouli oil - 1 drop ylang-ylang	Slowly added to a gold-based cream, left to rest, and then applied.
Body Odor	5% essential oil blend in hydrosol: -Patchouli, -Ylang Ylang	Apply once daily
Boil	- 40 ml olive oil - 4 drops oregano - 4 drops rosemary - 4 drops lavender - 7 drops chamomile - 4 drops peppermint	Applied once a day.
Bronchitis	- 100 ml jojoba oil - 10 drops cajeput - 10 drops niaouli - 10 drops myrtle	Applied by massaging the chest area.
Bronchitis (Child)	- 50 ml almond oil - 8 drops rosemary or ginger - 6 drops juniper oil - 7 drops peppermint or eucalyptus	Applied morning and evening.
Bronchitis (Baby)	- 6 drops lavandin - 2 drops vanilla - 100 ml almond oil	Applied by gentle massage.
Bronchitis (Dry Cough)	- 6 drops ginger - 4 drops juniper - 5 drops eucalyptus - 30 ml argan oil	Rubbed on the chest area.
Calluses Treatment	- 1 drop sage oil - 1 drop cypress - 1 drop Helichrysum - 1 drop Carpobrotus acinaciformis	Applied once a day.
	- 6% shea butter with - Oregano - Laurel - After the tissue has softened, Helichrysum is added	Applied once a day.
Cystitis	- 3 drops medical chamomile - 2 drops tea tree - 1 drop thyme (for pregnancy, use 1 drop tea tree)	Mixed in a glass of water and consumed.
Diaper Rash	- 30 ml chamomile water - 10 ml calendula - 10 drops medical chamomile	Applied twice a day.
Eczema - Itching	- 50 ml St. John's wort oil - 20 drops lavandin	Applied twice a day.
Eczema	- 1 ml palmarosa - 1 ml lavandin - 0.5 ml niaouli - 50 ml calendula oil	Applied twice a day.
Facial Paralysis	-50 ml rosehip oil -10 drops Rosemary cineole -5 drops Juniper -5 drops Marjoram	Apply with massage

Fibromyalgia	- 25 ml jojoba oil - 4 drops black pepper - 3 drops camphor - 4 drops sandalwood - 4 drops ylang-ylang	Applied by massage.
Haemorrhoid Treatment	- 3-4 drops chamomile EO in 1 liter of warm water - St. John's wort oil (topically)	Sitz bath with chamomile, topical application of St. John's wort.
Hair Loss	- 5 ml sesame oil - 5 ml castor oil - 5 ml almond oil - 2 ampoules containing dexpanthenol - 2 ampoules containing Vitamin E - 2.5 ml argan oil	Leave for 1 hour and wash.
Headache, Sinusitis, Cold	- 4 drops lavender essential oil - 4 drops peppermint essential oil - 2 drops frankincense essential oil	Applied using a diffuser.
Immune Boost	- 1 drop Helichrysum - 1 drop rose oil - 1 drop ginger - 1 drop thyme - 1g cinnamon	Taken internally.
Indigestion	- 6 drops chamomile - 6 drops lemon - 6 drops helichrysum - 30 ml coconut oil	Massaged onto the abdominal area.
Joint Pain	- 30 ml sweet almond oil - 30 ml apricot kernel oil - 10 ml jojoba oil - 8 drops lavender - 8 drops eucalyptus - 4 drops rosemary - 4 drops peppermint	Applied twice a day.
Lice	- 50 ml coconut oil - 7 drops lavender - 7 drops tea tree oil	Left on hair for 3-4 hours with a cap.
Menstruation (Regulation)	- 1 drop rose oil - 2 drops ylang-ylang - 2 drops myrtle - 2 drops grapefruit - 3 drops clary sage - 50 ml argan oil - 50 ml cod oil	Applied 3 times a day to the lower abdomen.
Menstruation (Pain Relief)	- 1 drop cedarwood - 2 drops ylang-ylang - 3 drops lavender - 2 drops myrtle - 2 drops jasmine - 70 ml sweet almond oil - 30 ml St. John's wort oil	Applied 3 times a day to the lower abdomen.
Nasal Congestion	- 10 drops eucalyptus in 1 liter of hot water	Inhaled as steam.
Neuropathic Pain	- 15 ml St. John's wort oil - 15 ml black cumin oil - 5 drops clove - 4 drops ginger - 6 drops black pepper - 3 drops rosemary - 2 drops wintergreen	Applied once a day.

Oily Hair	-9 drops Ylang Ylang -9 drops Lemon -8 drops Rosemary -50 ml grapeseed oil	Massage into scalp
Premenstrual Syndrome	- 6 drops palmarosa - 10 drops bergamot - 10 drops geranium - 4 drops clary sage - 50 ml sweet almond oil	Applied to lower back, tailbone, upper abdomen, and hips.
Rheumatoid Arthritis	- 2 drops peppermint - 2 drops wintergreen - 2 drops frankincense - 2 drops eucalyptus - 2 drops cypress - 30 ml coconut oil	Applied twice a day.
Scalp Dermatitis	- 10 ml St. John's wort - 10 ml calendula - 20 drops Helichrysum - 10 drops chamomile	Leave for 2 hours, then wash.
Shingles (*Herpes zoster*)	-100 ml rose or lavender hydrosol -5 drops Tea tree -2 drops Lemongrass -5 drops Lavender -3 drops Geranium	Spray 10 times, 5 times a day
Skin Discoloration (Spots)	- 3% calendula - Primrose - Avocado - Coconut - Cedarwood - Lemon - Bergamot - Helichrysum	Applied once a day.
Sleep Disorders	50 ml argan or jojoba oil -7 drops Lavandin -2 drops True Lavender -3 drops Palmarosa - 1 drop Lemongrass	Use with diffuser
Sore Throat, Hoarseness	- 2-3 drops tea tree oil in a glass of water	Used as a gargle 2-3 times a day.
Stretch Marks	- 10 drops frankincense - 5 drops geranium - 50 ml sweet almond oil	Applied once a day.
Sunburn	- 100 ml lavender or chamomile water - 10 drops tea tree oil - 5 drops medical lavender - 5 drops blue chamomile	Applied once a day.
Sweating	-5% sage essential oil in lavender hydrosol	Apply once daily
Ulcer	-1 drop Fennel -1 drop Rosemary -1 drop Lemon -1 drop Cumin	Take orally, morning and evening
Varicose Veins	-10 drops Geranium -15 drops Cypress -5 drops Lemon -30 ml carrier oil	Apply with massage

Table 2: The scientific names of the species, their commercial names, families and the values used in the aromatherapy prescriptions (**bold** species have the highest UV value).

Scientific names	Families	Commercial names	UV
***Sideroxylon spinosum* L.** **(Syn.:*Argania spinosa* (L.) Skeel)**	Sapotaceae	Argan	**0.16**
***Boswellia sacra* Flück.**	Burseraceae	Frankincense	0.048
***Calendula officinalis* L.**	Asteraceae	Calendula	0.048
***Cananga odorata* (Lam.) Hook.f. & Thomson**	Annonaceae	Ylang Ylang	**0.22**
***Carpobrotus acinaciformis* (L.) L.Bolus**	Aizoaceae	*Carpobrotus acinaciformis*	0.016
***Cedrus libani* A.Rich.**	Pinaceae	Cedarwood	0.048
***Chrysopogon zizanioides* (L.) Roberty**	Poaceae	Vetiver	0.016
***Camphora officinarum* Boerh. ex Fabr.**	Lauraceae	Camphor	0.016
***Cinnamomum verum* J.Presl**	Lauraceae	Cinnamon	0.016
***Citrus* × *limon* (L.) Osbeck** **(Syn.: *C.* × *bergamia* (Risso) Risso & Poit.)**	Rutaceae	Bergamot	0.03
***Citrus* × *limon* (L.) Osbeck**	Rutaceae	Lemon	0.079
***Citrus* × *aurantium* L.** **(Syn.: C. x *paradisi* Macfad.)**	Rutaceae	Grapefruit	0.016
***Cocos nucifera* L.**	Arecaceae	Coconut	0.03
***Cuminum cyminum* L.**	Apiaceae	Cumin	0.016
***Cupressus sempervirens* L.**	Cupressaceae	Cypress	0.048
***Cymbopogon citratus* (DC.) Stapf**	Poaceae	Lemongrass	0.03
***Cymbopogon martini* (Roxb.) Will.Watson**	Poaceae	Palmarosa	0.016
***Eucalyptus radiata* Sieber ex DC.**	Myrtaceae	Eucalyptus	0.079
***Foeniculum vulgare* Mill.**	Apiaceae	Fennel	0.03
***Gaultheria procumbens* L.**	Ericaceae	Wintergreen (Gaultheria)	0.03
***Helichrysum* Mill.**	Asteraceae	Helichrysum	**0.13**
***Hypericum perforatum* L.**	Hypericaceae	St. John's Wort	0.063
***Jasminum officinale* L.**	Oleaceae	Jasmine	0.016
***Juniperus communis* L.**	Cupressaceae	Juniper	0.048
***Laurus nobilis* L.**	Lauraceae	Laurel	0.016
***Lavandula angustifolia* Mill.**	Lamiaceae	Lavender	**0.32**
***Leptospermum scoparium* J.R.Forst. & G.Forst.**	Myrtaceae	Manuka	0.016
***Matricaria chamomilla* L.**	Asteraceae	Blue Chamomile	0.048
***Melaleuca alternifolia* (Maiden & Betche) Cheel**	Myrtaceae	Tea Tree	**0.13**
***Melaleuca cajuputi* Maton & Sm. ex R.Powell**	Myrtaceae	Cajeput	0.016
***Melaleuca quinquenervia* (Cav.) S.T.Blake**	Myrtaceae	Niaouli	0.03
***Mentha* × *piperita* L.**	Lamiaceae	Peppermint	0.095
***Myrtus communis* L.**	Myrtaceae	Myrtle	0.016
***Nigella sativa* L.**	Ranunculaceae	Black Cumin	0.016
***Oenothera biennis* L.**	Onagraceae	Primrose	0.016
***Olea europaea* L.**	Oleaceae	Olive	0.016
***Origanum majorana* L.**	Lamiaceae	Marjoram	0.016
***Origanum vulgare* L.**	Lamiaceae	Oregano	0.048
Pelargonium graveolens	Geraniaceae	Geranium	0.048
***Persea americana* Mill.**	Lauraceae	Avocado	0.016
***Piper nigrum* L.**	Piperaceae	Black Pepper	0.03
***Pogostemon cablin* (Blanco) Benth.**	Lamiaceae	Patchouli	0.03

***Prunus armeniaca* L.**	Rosaceae	Apricot	0.016
***Prunus amygdalus* Batsch**	Rosaceae	Sweet Almond	0.03
***Ricinus communis* L.**	Euphorbiaceae	Castor Oil	0.03
***Rosa canina* L.**	Rosaceae	Rosehip	0.016
***Rosa* × *damascena* Herrm.**	Rosaceae	Rose	0.079
***Salvia rosmarinus* Spenn.**	Lamiaceae	Rosemary	**0.17**
***Salvia officinalis* L.**	Lamiaceae	Sage	0.063
***Salvia sclarea* L.**	Lamiaceae	Clary Sage	0.016
***Santalum albüm* L.**	Santalaceae	Sandalwood	0.016
***Sesamum indicum* L.**	Pedaliaceae	Sesame	0.016
***Simmondsia chinensis* (Link) C.K.Schneid.**	Simmondsiaceae	Jojoba	0.079
***Syzygium aromaticum* (L.) Merr. & L.M.Perry**	Myrtaceae	Clove	0.016
***Thymus vulgaris* L.**	Lamiaceae	Thyme	0.048
***Vanilla planifolia* Andrews**	Orchidaceae	Vanilla	0.016
***Vitellaria paradoxa* C.F.Gaertn.**	Sapotaceae	Shea Butter	0.016
***Vitis vinifera* L.**	Vitaceae	Grape	0.016
***Zingiber officinale* Roscoe**	Zingiberaceae	Ginger	0.063

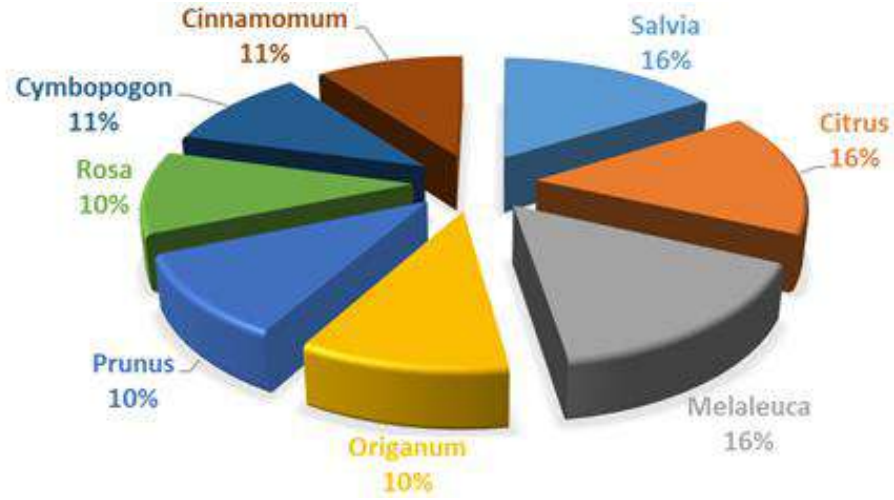

Figure 5: The genera represented by the most species in the prepared recipes.

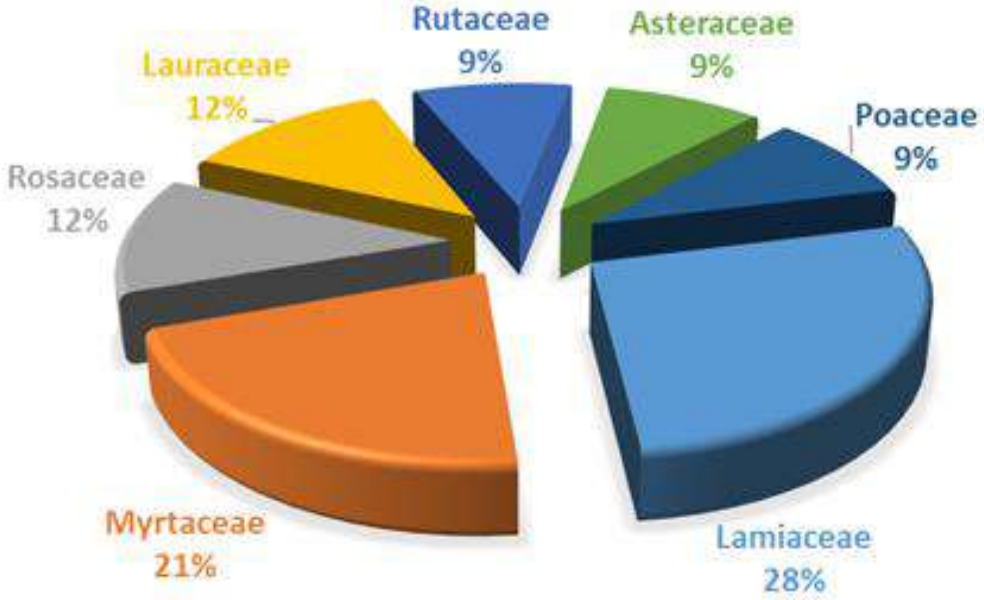

Figure 6: Families represented by the most genera in the prepared recipes.

DISCUSSION

In this study, medicinal plants included in prescriptions written by health professionals within the scope of aromatherapy applications in Türkiye were examined and evaluated in terms of species diversity, usage values (UV), functional consensus index (FIC) and therapeutic contents. The findings reveal that certain plants stand out in aromatherapy applications both culturally and therapeutically. For centuries, essential oils have been valued not only for their pleasant scents but also for the healing effects they provide on the body, mind and soul. The powerful plant compounds found in these oils have the ability to purify the environment from diseases, bacteria, viruses and fungi (Baratta et al., 1998). Essential oils offer multifaceted benefits such as strengthening the immune system, supporting hormonal balance, providing emotional balance, improving blood circulation, calming, strengthening memory and increasing mental alertness, as well as having antibacterial, antiviral and anti-inflammatory effects. These properties have been supported by many scientific studies and pilot projects (Scheau et al., 2024; Gokkaya et al., 2024).

First of all, the high UV values of the most frequently used plants in prescriptions, such as *Lavandula angustifolia, Matricaria chamomilla*, *Salvia rosmarinus* and *Eucalyptus globulus*, indicate that these plants are widely preferred both in traditional knowledge and in modern aromatherapy approaches. This finding has been reported in other ethnobotanical studies (Heinrich et al., 2006; Orhan et al., 2010). The high UV values reflect a consensus of preference based on common knowledge and experience between users and health professionals. This also suggests that the pharmacological efficacy of these species may have been observed in practice and therefore integrated into clinical decision processes.

The FIC values obtained in the study also support this view. The high level of FIC value of 0.87, especially in the respiratory diseases category, shows that there is a strong consensus on the plants used in this area. This situation shows that species rich in volatile compounds such as *Eucalyptus globulus, Mentha piperita* and *Thymus vulgaris* are effectively used in practice for their antimicrobial, expectorant and anti-inflammatory effects (Panchal et al., 2024; Ahsan et al., 2024). On the other hand, the lower FIC values in some usage categories reveal that there is more variety or uncertainty in prescriptions for these areas, and this situation shows that more clinical

studies and standardization are needed in the relevant areas.

Chemical component analyses show that the species preferred in aromatherapy largely contain well-characterized therapeutic constituents (Sadgrove et al., 2022; Bunse et al., 2022). For example, components such as linalool, 1,8-cineole, menthol and thymol stand out in aromatherapy with their sedative, bronchodilator, antimicrobial and anti-inflammatory effects. The presence of these compounds shows that aromatherapy is not only a traditional or complementary practice, but also has a pharmacological basis (Kurt and Cankaya, 2021; Stojanović et al., 2024; Yenikalayci et al., 2025).

Essential oils are complex mixtures of volatile compounds that are important for the plant itself and for people who have learnt how to use them over the years. According to ISO norm 9235:2013, an essential oils is a "product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase—if any—by physical processes" (ISO NORM 2013). The European Pharmacopoeia defines an essential oils as "an odorous product, usually of complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating." A physical process that doesn't change the composition of essential oils is usually used to separate them from the aqueous phase (EDQM 2021). It is clear from both definitions that only products made by steam or hydrodistillation can be called essential oils. Products made by other extraction methods that use solvents must be called extracts. Essential oils are primarily distinguished by the presence of terpenes/terpenoids and phenolic compounds (specifically, phenylpropanoids), which originate from the mevalonate/methyl erythritol and shikimic acid pathways, respectively. The term "100% natural," when used (and verified) in accordance with standards like ISO, indicates that there are no synthetic additives/chemical additions; it creates the perception of "chemical-free" for consumers. However, in practice, there may be compounds that are natural but can be toxic or allergenic; the misconception that "natural = safe" is risky (Iheukwumere et al. 2025). The term "100% holistic" means that the product has not been subjected to any treatments that would destroy the molecules. This may emphasize holistic, ethical, and sustainable methods (e.g., organic farming, fair trade, ritual practice) in terms of production, harvesting, processing, and perhaps application. However, this definition must also be

documented with evidence-based experimental reports. In the literature, the chemical composition of an essential oils are usually expressed as relative percent abundance (i.e., % area) or normalized percent abundance (i.e., normal % area) (Bicchi et al. 2008). This process is arduous and time-consuming, so few articles have accurately measured EO. Quality control of EO is important to ensure its safety. Adding cheaper EOs to EOs (e.g., adding bitter orange to sweet orange, peppermint to mint, or lavandin to lavender sugar) or adding cheap synthetic ingredients (e.g., adding synthetic linalool and linalyl acetate to bergamot EO) can often make the EO less pure. The term "100% pure" means that the product contains no other vegetable oils, diluents, or synthetic compounds. However, this statement alone has limited commercial validity; GC-MS, enantiomeric analysis, and other analytical methods are required (Gharibzahedi and Altintas 2025). Such degradation can be easily detected by looking at the normalized percent area of specific markers. Furthermore, because biosynthesis in plants is driven by stereochemistry, and terpenes and terpenoids generally have a specific enantiomeric composition, it can be used to detect whether essential oils have been degraded by the addition of synthetic volatile compounds (Capetti et al. 2021).

This study also reveals that aromatherapy has gained increasing interest among the public in Türkiye and has begun to be integrated into clinical practices. However, the contextual analysis of prescriptions also shows that this integration is still lacking a systematic approach (da Silva-Brandao et al., 2023). Parameters such as clinical effects, dosage information, interactions and patient profiles are often not included in prescriptions, which raises the issue of lack of standardization in aromatherapy practices (Brennan et al., 2022; Vora et al., 2024; Silva, 2025). In this context, structuring aromatherapy prescriptions in light of scientific evidence-based guidelines will both increase therapeutic efficacy and reduce possible toxicological risks.

In conclusion, this study is one of the rare examples of systematic evaluation of prescribed aromatherapy practices in Türkiye from botanical, ethnobotanical and therapeutic perspectives. The findings show that both folk medicine tradition and modern medical approaches come together in aromatherapy. In future studies, follow-up studies observing the clinical effects of these prescriptions will provide important contributions to public health policies and complementary medicine practices.

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Phytochemicals, Green Synthesis of Copper and Silver Nanoparticles from *Plectranthus amboinicus* Leaf Extract and Evaluation of Antioxidant and Antibacterial Activities

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Abstract

Plectranthus amboinicus belongs to the Lamiaceae family and it is used in folk medicine for treatment of epilepsy, pneumonia and flu. The study aimed to investigate the phytochemicals and to green synthesize nanoparticles and to evaluate the antioxidant and antibacterial activities. The macroscopic, microscopic and physicochemical parameters of the leaves were analyzed and subsequently extracted in 80% ethanol and distilled water by maceration. Copper (Cu) and silver (Ag) nanoparticles were synthesized via green methods using 5 mM $CuSO_4$ and $AgNO_3$ respectively. The nanoparticles were characterized using Zeta analyser (size and potential), UV and IR. The antioxidant activity was assessed using DPPH free radical with vitamin C as reference. The antibacterial activity was determined using micro broth dilution (MIC) and subculturing (MBC) on *E. coli* and *S. aureus*. The physicochemical studies revealed. Moisture content (13.1% ± 1.86 S.D), total ash value (16.9% ± 0.98), acid insoluble ash value (0.7% ±0.55, hexane soluble extractive value (9.45% ± 1.79), alcohol soluble extractive value (2.03% ± 0.80) and water-soluble extractive value (1.63% ± 0.30). The phytochemical screening revealed the presence of tannins and flavonoids. The antioxidant studies revealed IC_{50} in mg/mL for water extract (1.245), ethanol extract (11.26), CuNPs (5.857), AgNPs (0.4272) and vitamin C (0.3245). The copper nanoparticles produced MIC of 1.25 mg/mL and 0.625 mg/mL on *E. coli* and *S. aureus* respectively. The water and ethanol extracts produced MIC of 10 to 5 mg/mL respectively on *E. coli* and *S. aureus*. The copper nanoparticles had MBC of 10 mg/mL on *E. coli* and 5 mg/mL *S. aureus*. The findings indicate that *P. amboinicus* contains phytochemicals with antioxidant and antibacterial properties, which supports its use in traditional medicine to prevent and treat illnesses linked to free radicals and bacterial infections.

Keywords

Antioxidant, *plectranthus amboinicus*, physicochemical, phytochemical.

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INTRODUCTION

Plant secondary metabolites are produced as defense chemicals and have been used to treat human diseases thus playing a crucial role in maintaining health (Altemimi, 2017). In recent years, extensive researches have been conducted on potential health benefits of phytochemicals with distinct bioactivities (Dillard, 2000; Arora, 2020).

Plectranthus amboinicus (Lour.) Spreng also known as Indian borage is a perennial herb from the Lamiaceae family that grows naturally in the tropical and warm regions of Africa, Asia, and Australia (Arumugam, 2016). *P. amboinicus* is a luscious, meaty herb well-known for its distinct oregano flavored scent and the indigenous populations of tropical forests use it extensively for culinary purposes (Arumugam, 2016). In folk medicine, it is common to extract the juice from the leaves and mix it with honey in a hot decoction for treatment of colds and coughs (Arumugam, 2016). The juice also acts as a powerful carminative when mixed with sugar (Sahu, 2022).

P. amboinicus have been used for its medicinal and nutritional values and this has been ascribed to its valuable phytochemicals (Satheesh, 2022).

P. amboinicus is an annual herbaceous plant with a characteristic odour and traditional medicine, its crenate-shaped leaves with serrated edges are frequently used to treat cough, asthma, pneumonia, flu, fevers and epilepsy. These biological activities have been linked to the presence of flavonoids such as apigenin, luteolin and salvigenon, tannins and saponins (Asaduzzaman, 2018; Satheesh, 2022).

In Comoros, the leaves are highly valued for their scent, and are commonly found in gardens and are widely used as tea.

Numerous phytochemical classes, including monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols, and aldehydes have been identified in *P. amboinicus* (Arumugam, 2016).

The essential oils are rich in bioactive compounds, primarily monoterpenoids such as thymol, carvacrol, α-terpineol, γ-terpinene, and ρ-cymene, with a variety of pharmacological effects (Ashaari, 2021).

P. amboinicus volatile oils have demonstrated antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermis* and *S. aureus* (Erny Sabrina, 2014). Furthermore, the oils have demonstrated antifungal against *Aspergillus flavus*, *Aspergillus niger*, *Penicillium sp*., (Murthy, 2009). This study seeks to explore the phytochemicals of the leaves extracts and to green synthesize phyto-nanoparticles using

copper and silver salts with the view of investigating their antioxidant and antibacterial activities.

MATERIALS AND METHODS

Identification, collection and preparation of *P. amboinicus*

The fresh plants were collected at a home vegetable garden in Comoros, East Africa. The identity of the plant was authenticated by Asst. Prof Dr. Emmanuel Mshelia Halilu, at Cyprus international University, Faculty of Pharmacy, Department of Pharmacognosy. A voucher specimen with number CIU/PHARM/LAMI/010 was prepared and then deposited at the herbarium of the faculty for reference. More of the leaves were collected and then air dried under the shade for one week and then powdered using a blender. The resulting powder was stored in an airtight container with label until required for further usage.

Macroscopic and microscopic evaluation *P. amboinicus* powder leaves

The methods outlined by Halilu *et al*. (2008) and Atli *et al*. (2024) were used. The sense organs were used for the macroscopic evaluations. For the microscopic evaluation, the powder leaves were boiled in chloral hydrate for 15 minutes to remove chlorophyll and other obscuring material. The cleared powder was transferred to a glass slide with the aid of forceps, then followed by addition of one drop dilute glycerol. The cover slip was placed and excess glycerol was removed with the aid of blotting paper. The prepared slide was placed under the microscope and then observed at x10 and x40 objectives for the presence of trichomes, calcium oxalates and other microscopic characters.

Physicochemical studies

The methods outlined by Halilu *et al*. (2008) were followed for the determination of the physicochemical parameters.

Moisture content (MC)

The loss of weight on drying method was used. The powder leaf (1g) was measured and then transferred to an empty crucible. This was heated in an oven at 105°C for 1 hour and then removed. It was allowed to cool in a desiccator and the weight was measured. This was placed in the oven and weight was taken after every 30 minutes until a constant weight was obtained from the last two consecutive readings. The experiment was conducted in triplicate and the percentage moisture content was calculated as follows:

$$\%\ \text{Moistue content} = \frac{\text{Weight of moisture}}{\text{Initial weight of sample}} \times 100$$

Total ash value (TAV)

The total ash value was estimated by weighing 1 g of powder leaf into a crucible and burn in a furnace set from 0°C to 550°C for a period of 30 minutes and the then allowed to burn for 3 hours until the plant tissues were transformed to ashes. After three hours of burning the temperature of the furnace was allowed to gradually decrease to 0°C within 1 hour. The experiment was conducted in triplicate and the percentage total ash value was computed as follows:

$$\%\ \text{Total Ash Value} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Acid insoluble ash

The total ash obtained was dissolved in 30 mL of 2N of HCl and then filtered gradually using a pre-weighed filter paper. After the filtration, the paper with the insoluble ash were placed in an oven at 33 °C for 72 hours and the weight was measured. The experiment was conducted in triplicate and the percentage acid insoluble was estimated as follows:

$$\%\ \text{Acid Insoluble Ash} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of sample}} \times 100$$

Hexane, alcohol and water extractive values

The extractive values for each experiment was determined by weighing 1g of the powder plant into a conical flask and then macerating in 30 mL of each solvent for 24 hours. They were filtered separately and then 15 mL of the filtrates were transferred to a pre-weighed crucibles and then left to dry for 72 hours at 33°C. The experiments were conducted in triplicates each and the percentage extractive values were determined as follows:

$$\%\ \text{Extarctive values} = \frac{\text{Weight of extract}}{\text{Weight of plant sample}} \times 100$$

Extraction of plant powder in 80% ethanol and distilled water

The plant powder (5 g) was weighed in conical flask and then extracted by maceration in 100 mL of 80% ethanol with stirring on a magnetic stirrer for 24 hours. It was then filtered and the marc was washed with 150 mL of the extracting solvent. The filtrate obtained was then concentrated in a rotary evaporator 50 °C for 25 minutes at 40 rpm. The remaining concentrated extract transferred to an evaporating dish to dry at 33 °C 72 hours. The same procedure was repeated for the water extract and the percentage yield was determined thus:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.5)$$

Qualitative phytochemical screening

The qualitative phytochemical analysis for the determination of saponins, steroids/triterpenoids, tannins, flavonoids and alkaloids were determined according the methods outlined by Halilu *et al*. (2008) and Atli *et al*. (2024).

Green synthesis of copper and silver nanoparticles

The synthesis was carried out according to the protocol of Halilu *et al*. (2024). The precursors used for the synthesis were copper sulphate ($CuSO_4$) and silver nitrate ($AgNO_3$). The 5 mM solution of CuSO4 was prepared by dissolving 0.322g of copper sulphate in 400 mL of distilled water with stirring it for a few minutes using a magnetic stirrer. The silver nitrate solution was prepared by dissolving 0.254g of silver nitrate in 400 mL and stirring of distilled water with stirring for a few minutes using a magnetic stirrer. The salt solutions were mixed with the plant extracts in the ratio of 9:1 respectively with constant stirring at 250 rpm for 24 hr. After 24 hours of stirring, the solution was kept in the dark for a further 24 hours in the dark. After the formation of the particles, they were centrifuged at 4100 rpm for at least 8 to 10 minutes. The supernatant was decanted and the nanoparticles was transferred to petri dishes and then allowed to dry. The particles were scraped using a blade, and were stored for further uses.

Characterization of the silver and copper nanoparticles

The characterization of the nanoparticles was carried out as outlined by Halilu *et al*. (2023).

UV spectra

The supernatant of the nanoparticle was transferred into a cuvette and then analyzed using the UV spectrophotometer between wavelengths of 200-800 nm where the absorbance was measured.

Zeta analysis

The size and potential of the copper and silver nanoparticles were analyzed using the zeta sizer, where the supernatant was placed in a cuvette and then analyzed at room temperature (about 25°).

IR analysis

The copper and zinc nanoparticles were analyzed for the presence of some functional groups using the FTIR machine. The samples were scanned between the range of 4000-400 cm^{-1} and transmittance was obtained.

Antioxidant activity using DPPH free radical

The methods outlined by Halilu *et al*. (2023) were followed to determine the antioxidant activity of the 80% ethanol, water extract, nanoparticles and the

ascorbic acid using DPPH free radical scavenging activity.

Antibacterial activity of extracts and nanoparticles

The bacteria strains of *Staphylococcus aureus* (MRSA) and *Escherichia coli* O157:H7 (932) were obtained from the Microbiology Laboratory, Cyprus International University. The agar was prepared as specified by the manufacturer. The zone of inhibition of 80% ethanol extract, water extract, copper and silver nanoparticles were determined using the disk diffusion method and ciprofloxacin was used as the positive control (Emmanuel *et al*., 2023). The minimum inhibitory concentration (MIC) of the test samples on the bacteria was determined using the microdilution assay using the 96 well plates. The minimum bactericidal concentration (MBC) was obtained by sub-culturing.

RESULTS

Macroscopic and microscopic evaluation

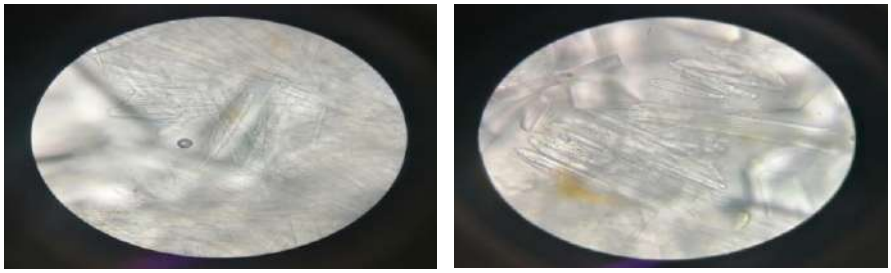
The organoleptic characters of *P. amboinicus* are shown in Table 1. The microscopic features showing prismatic, raphides type of calcium oxalate crystals and trichomes at x10 and x40 objectives are presented in Figures 1, 2 and 3 respectively.

Table 1: Organoleptic evaluation of *P. amboinicus.*

Characteristic	Observation
Smell	Strong
Texture	Very bitter
Taste	Greenish brown
Color	Coarse

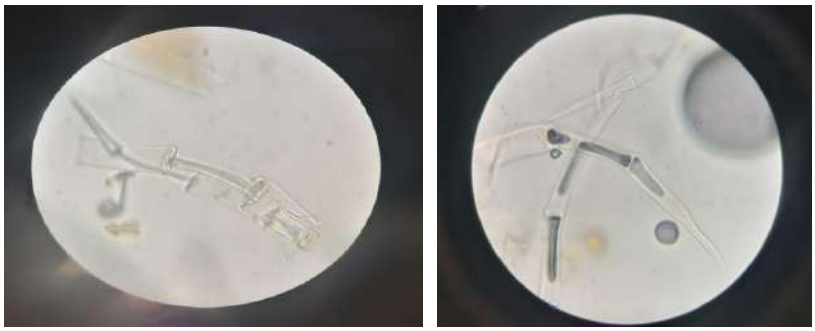
(a) **(b)**

Figure 1: Microscopic view of the prismatic calcium oxalate crystals (a) x10 & (b) x40.



(a) **(b)**

Figure 2: Microscopic view of the raphides type of calcium oxalate (a) x10 & (b) x40**.**



(a) **(b)**

Figure 3: Microscopic view of covering trichomes at (a) x10 & x40.

Physicochemical evaluation

The moisture content, total ash value, acid insoluble ash value, hexane soluble extractive value, alcohol soluble extractive value, and water-soluble extractive value are presented in Table 2.

Table 2: Physicochemical parameters of *P. amboinicus* powder.

Parameters	%Results (Means ± SD)
Moisture content	13.1% ± 1.86
Total Ash Value	16.9% ± 0.98
Acid Insoluble Ash Value	0.7% +/- 0.55
Hexane Soluble Extractive Value	9.45% ± 1.79
Alcohol Soluble Extractive Value	2.03% ± 0.80
Water Soluble Extractive Value	1.63% ± 0.30

Extraction and phytochemical screening

The percentage yield of the extract in 80% ethanol and water was 13.0 % and 23.24 % respectively. The class of secondary metabolites present the aqueous extract is shown in Table 3.

Table 3: Phytochemical screening of *P. amboinicus* extracts.

Metabolite/Test	Observation	Inference
Saponins/Frothing test	No Froth formed	-
Phenolic group/ferric chloride	Greenish precipitates formed	+
Tannins/Lead acetate	Cream precipitate formed	+
Flavonoids/ sodium hydroxide	Deep yellow coloration	+
Steroid and triterpenoids/Salkowski's	No reddish ring was observed	+
Alkaloids/Dragendorff's	No orange precipitate formed	-
/Mayer's	No creamy precipitate formed	-
/Wagner's	No brown precipitate formed	-
/Tannic acid	No brownish precipitate formed	-
/Hager's	No Yellow precipitate formed	-

Synthesis of copper and silver nanoparticles

The visual observation of the reduction of the Cu^{2+} ions to Cu^{o} is shown in Figure 4. The reduction of Ag^{+} to Ag^{o} is shown in Figure 5. The copper and silver nanoparticles produced are seen in Figure 6.

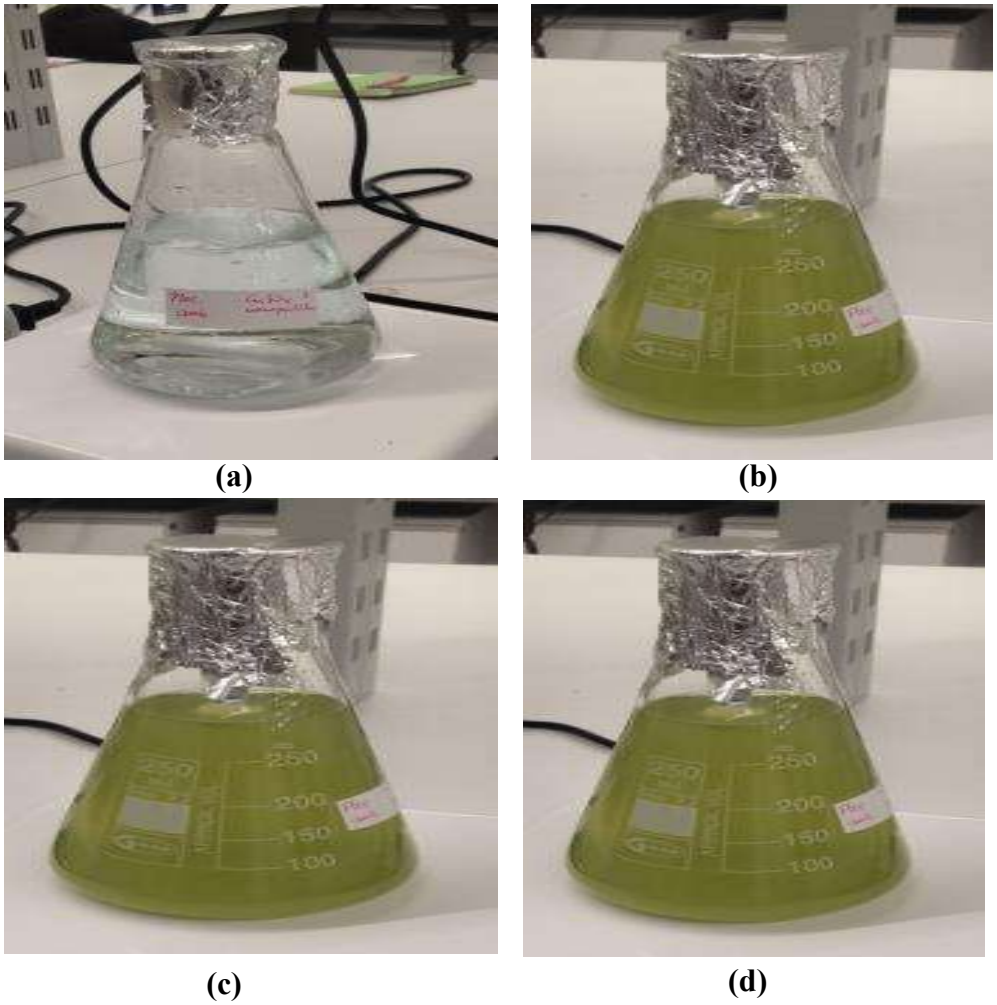


Figure 4: (a) $CuSO_4$ solution, (b) Copper nanoparticles + 80% Ethanol extract, (c) Copper nanoparticles after 1 hour of stirring, (d) Copper nanoparticles kept in the dark for 24 hours.

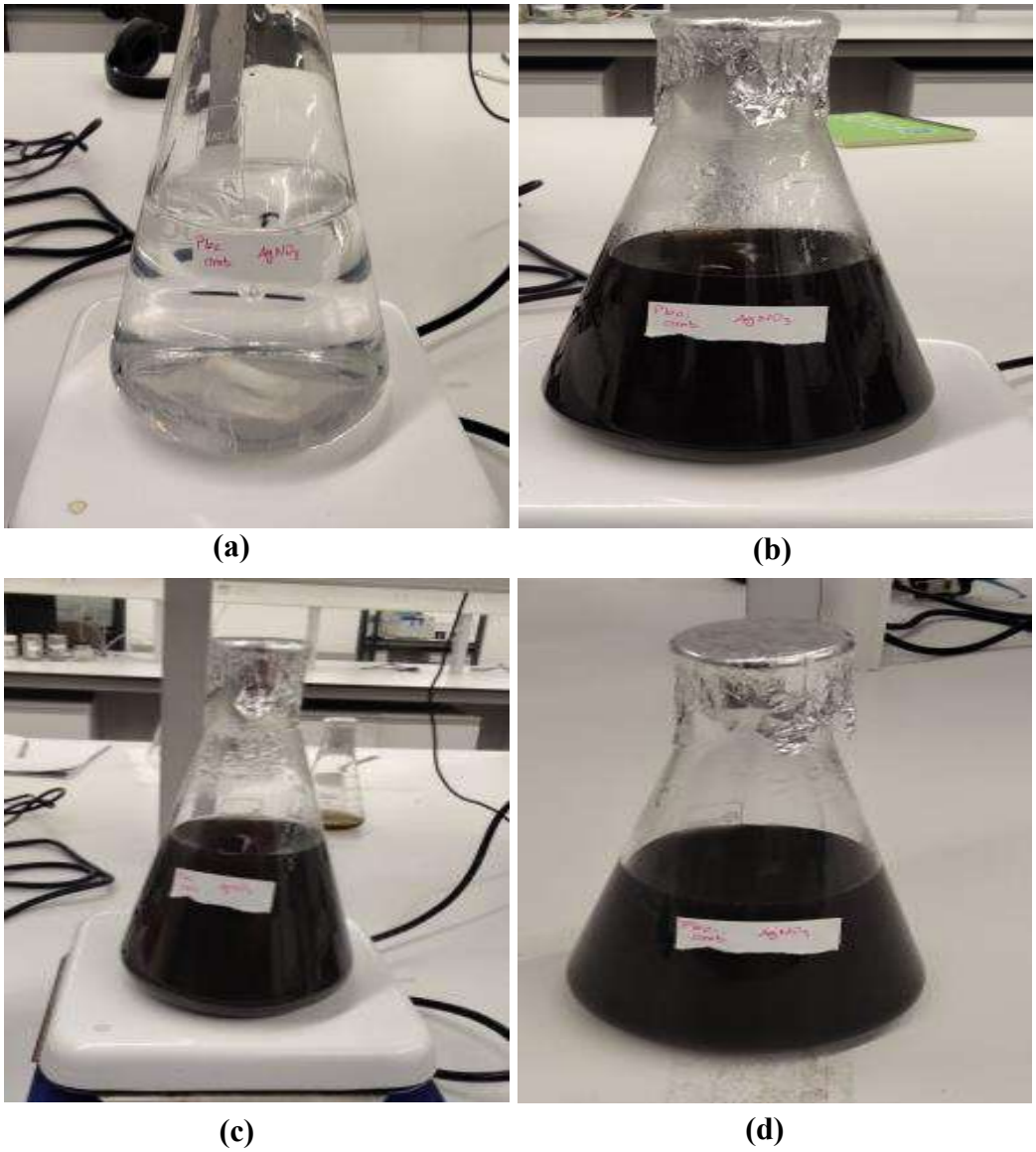


Figure 5: (a) $AgNO_3$ solution, (b) Silver nanoparticles + 80% Ethanol extract, (c) Silver nanoparticles after 1 hour (d) Silver nanoparticles kept in the dark for 24 hours.

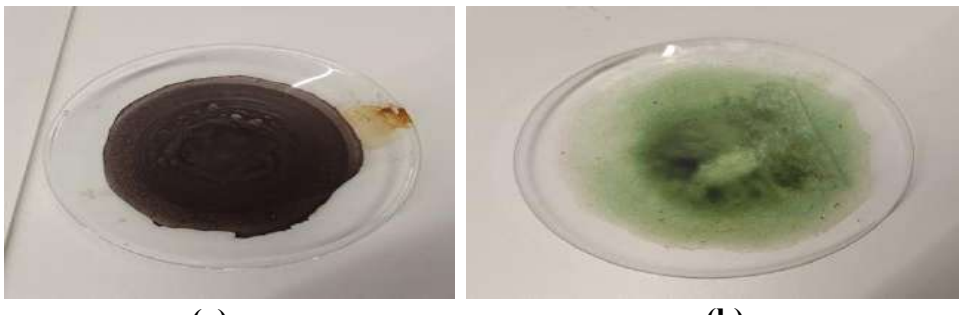


Figure 6: (a) Copper nanoparticles after drying & (b) Silver nanoparticles after drying.

Characterization of the nanoparticles

UV spectroscopy

The UV spectrum of the copper nanoparticles exhibit characteristic absorptions at wavelengths of 219.0 nm, 207.5 nm, and 223.5 nm. The silver nanoparticles exhibit characteristic absorptions at wavelengths of 228.5 nm, 212.0 nm, 226.5 nm, and 215.0 nm.

Zeta analysis

The size distribution of the copper nanoparticles was 100 nm (Figure 7) and potential of -23 mV (figure 8). The size distribution of the silver nanoparticles was 180 nm (Figure 9) and potential of 15 mV (figure 10).

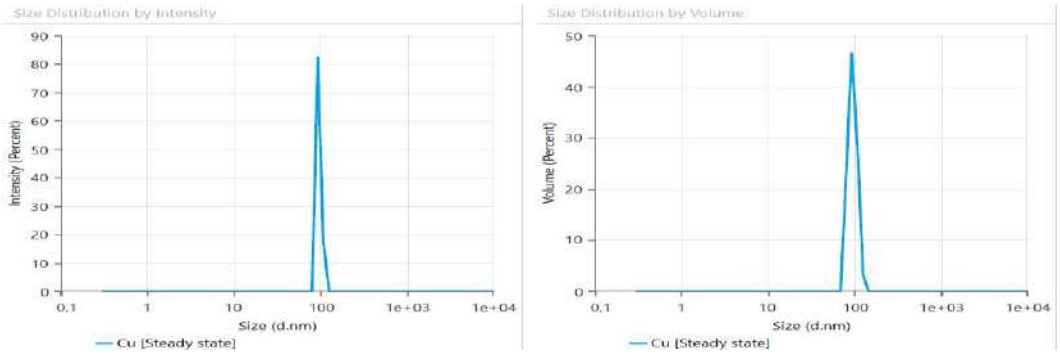


Figure 7: Zeta Size for Copper nanoparticles.

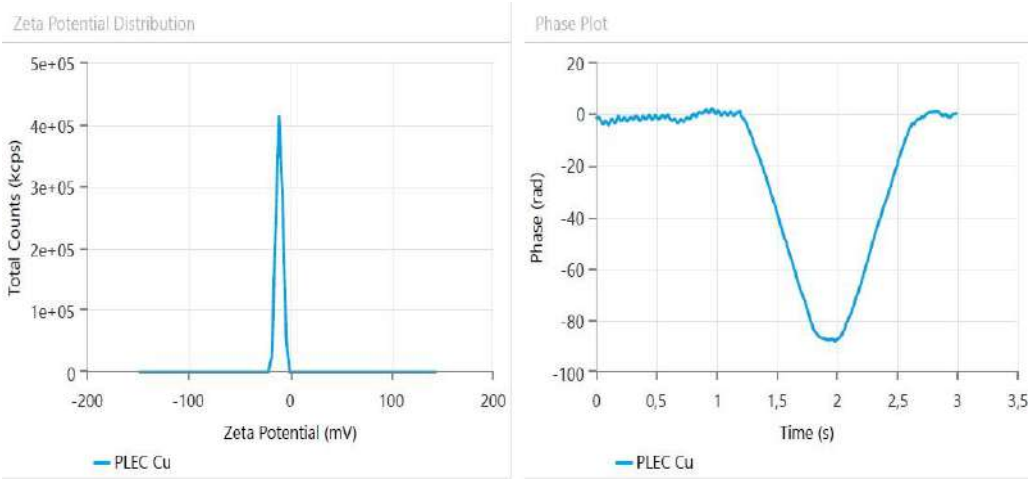


Figure 8: Zeta Potential for Copper nanoparticles**.**

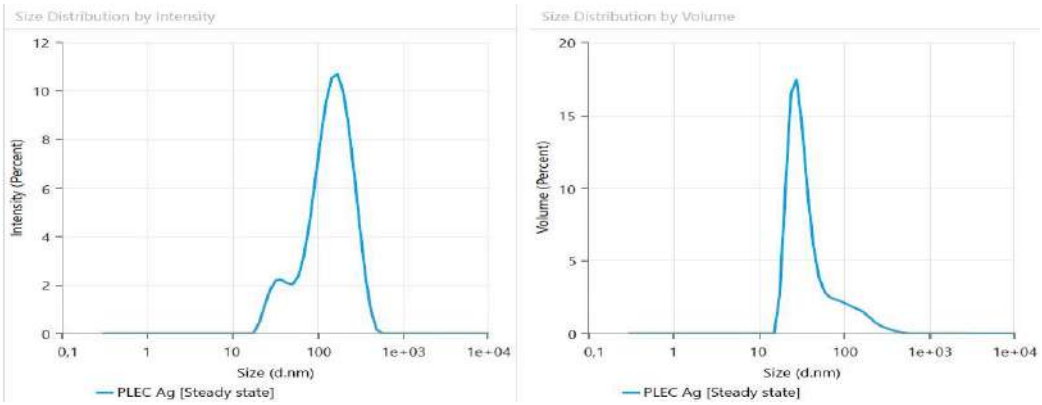


Figure 9: Zeta Size for Silver nanoparticles.

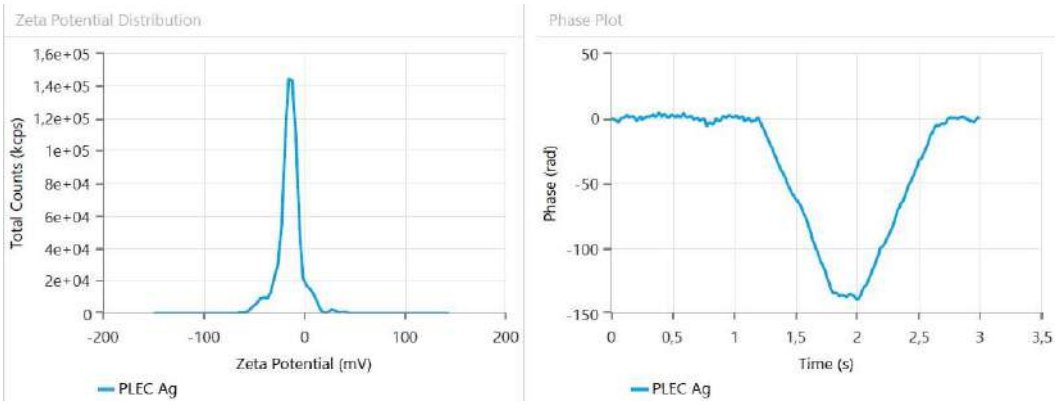


Figure 10: Zeta Potential for Silver nanoparticles**.**

IR spectrometry

The IR spectrum of **c**opper nanoparticles showed characteristic peaks at 3500 cm^{-1}, 3000 cm^{-1} and 1000 cm^{-1} which are typical of OH (broad), aliphatic primary amine NH (stretch), and CN stretch respectively. The silver nanoparticles showed characteristic peaks at 3500 cm^{-1}, 3000 cm^{-1} and 1250 cm^{-1} which are typical of OH (broad), aliphatic primary amine NH (stretch) and CN stretch respectively.

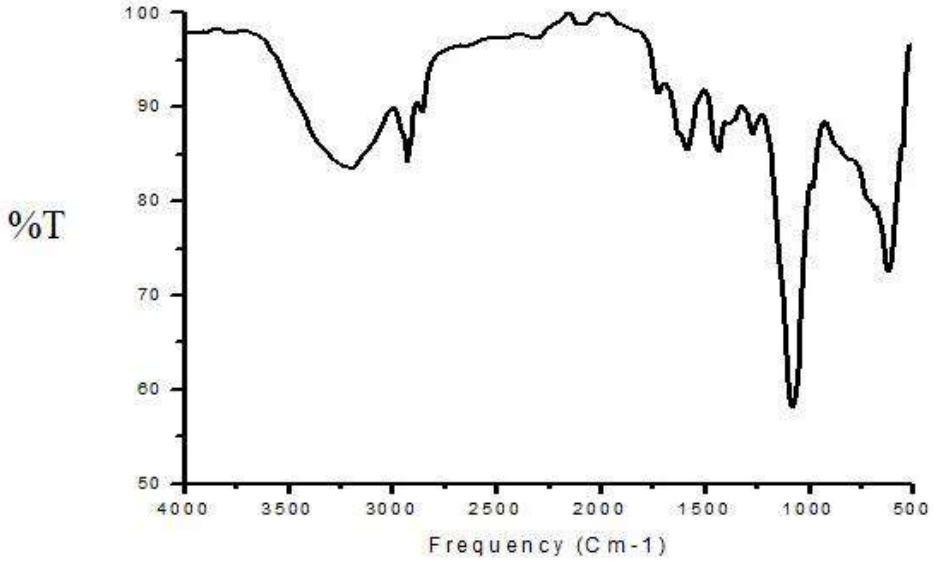


Figure 11: IR spectrum of the copper nanoparticles**.**

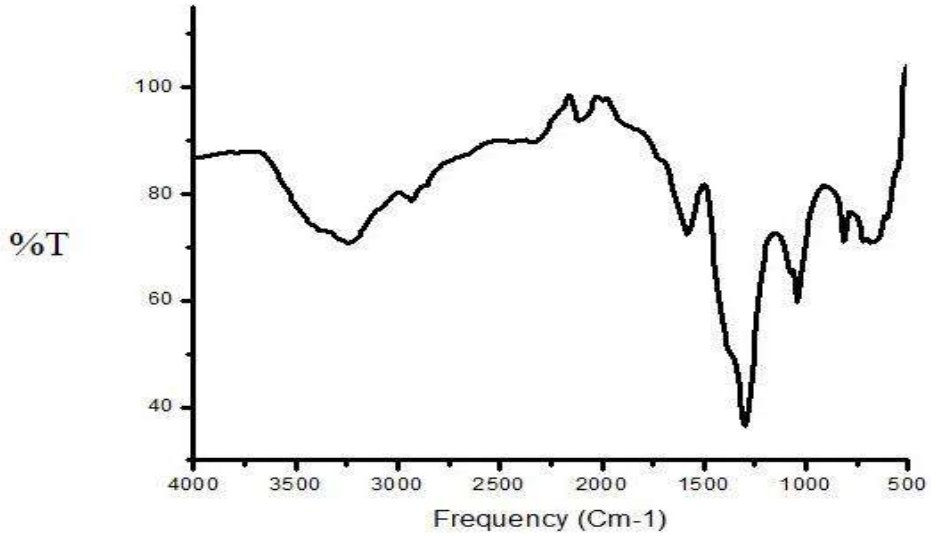


Figure 12: IR spectrum of the silver nanoparticles.

Antioxidant activity

The antioxidant activity of the samples is expressed in terms of percentage inhibition and IC_{50} values in mg/mL.

Table 4: % Inhibition and IC50 values samples.

Conc Mg/mL	Water extract	Ethanol extract	CuNPs	AgNPs	Vitamin C
0.625	73.53	68.66	-	-	74.14
1.25	73.79	67.52	26.17	26.17	58.46
2.5	88.11	60.93	20.30	28.18	68.75
5	99.46	60.80	18.28	30.53	55.10
10	55.45	86.08	61.07	32.71	52.54
IC_{50} mg/mL	**1.245**	**11.26**	**5.857**	**0.4272**	**4.363**

Antibacterial activity

There was no observed zone inhibition in the disk diffusion assay. However, in the MIC, the copper nanoparticles had MIC of 1.25 mg/mL and 0.625 mg/mL on *E. coli* and *S. aureus* respectively. The water and ethanol extracts produced MIC of 10 to 5 mg/mL respectively on *E. coli* and *S. aureus* (Figure 13).

The MBC showed that the copper nanoparticles at higher concentration was able to kill *E. coli* and *S. aureus* at 10 mg/mL and 5 mg/mL respectively (Figure 14). The 80% ethanol and water extracts did not kill the microorganisms. However, at 10 mg/mL 80% ethanol extract showed a positive response against *S. aureus* (Figure 15).

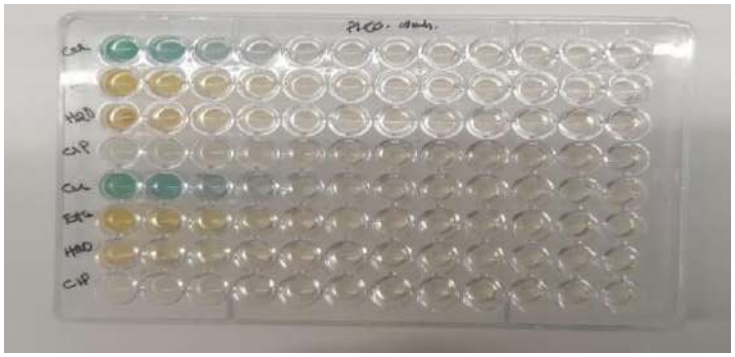


Figure 13: MIC sample 96 well plate.

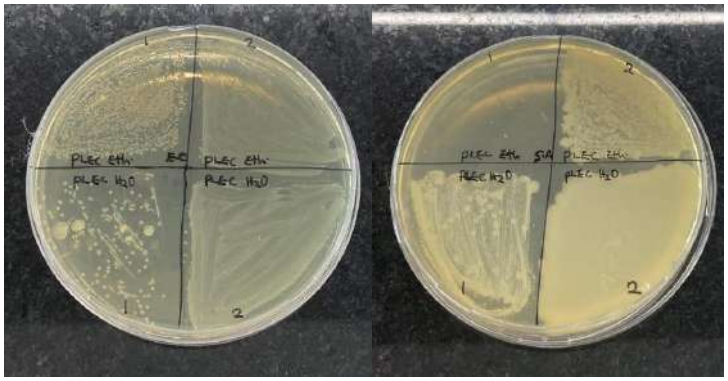


Figure 14: MBC sample plates for water and ethanol extracts *E. coli* and *S. aureus.*

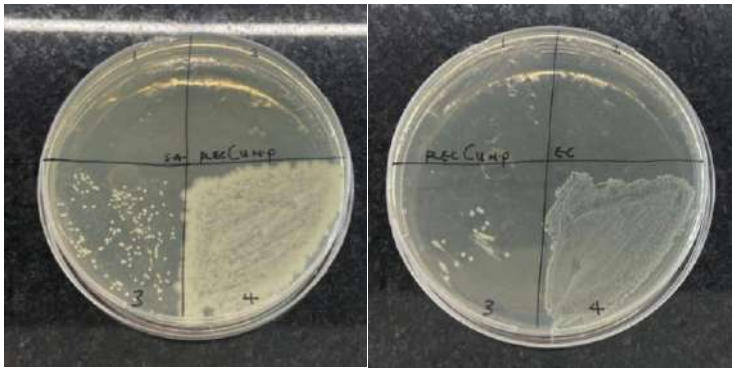


Figure 15: MBC sample plates of copper nanoparticles on *E. coli* and *S. aureus.*

DISCUSSION

The botanical evaluation of crude drugs has been recommended by the WHO (2011) as one of the first step towards the investigation of medicinal plants for phyto-therapeutic applications. The organoleptic evaluation showed that *P. amboinicus* powder has aromatic smell, greenish-brown colour, coarse texture and possesses a bitter taste. These characteristics agrees with the findings of Santos Filipe *et al*. (2025). The microscopy of the powder revealed the presence of covering trichomes, raphide and prismatic types of calcium oxalate crystals. The physicochemical studies revealed moisture content of 1.86 ± 13.1%, total ash value of 0.98 ± 16.9% and acid-insoluble ash value of 0.55 ± 0.7%. The extractive value revealed hexane soluble extract value of 1.79 ± 9.45%, alcohol soluble extract value of 0.80 ± 2.03% and water-soluble extract value of 0.30 ± 1.63%. On comparing the current findings on the physicochemical evaluation with Patel, (2010), and Pillai *et al*. (2011), their results are found to be higher. This difference may be due geographical location, soil chemistry and general variation in the environmental conditions. Higher moisture content encourages the growth of microbes and can lead to deterioration of the crude drug.

The percentage yield of the 80% Ethanol and water extract were found to be 13% and 23.24%, respectively. The water extract had the highest percentage yield and this may be attributed to its polarity. The phytochemical analysis revealed the presence tannins and flavonoids, The phytochemical screening result of the current study agrees with (Asiimwe, 2014). Although, they reported the presence of saponins which was absent in the current study. The presence of alkaloids has been reported by Patel (2010) but have not been detected in the current research. The presence of flavonoids has been reported by Ruan (2019) in *P. amboinicus*.

The antioxidant activity revealed IC_{50} of 1.245 mg/mL for the water extract and for the 80% ethanol extract it was 11.26 mg/mL and the vitamin C had 4.363 mg/mL. The result revealed that the water extract demonstrated the highest antioxidant activity by scavenging the DPPH free radical with the least IC_{50}. The results agree with the findings of Patel (2010) on *P. amboinicus* which has been shown to have significant antioxidant properties. The silver and copper nanoparticles revealed IC_{50} of 5.857 mg/mL and 0.4272 mg/mL respectively. The IC_{50} of vitamin C was 0.3245 mg/mL and compares well with the IC_{50} of copper

nanoparticles and this agrees with Revathi (2023).

The copper nanoparticles demonstrated antibacterial activity with MIC of 1.25 mg/mL and 0.625 mg/mL on *E. coli* and *S. aureus* respectively. The water and ethanol extracts produced MIC of 10 mg/mL and 5 mg/mL on *E. coli* and *S. aureus* respectively. The MBC showed that the copper nanoparticles at higher concentration was able to kill *E. coli* and *S. aureus* at 10 mg/mL and 5 mg/mL respectively.

CONCLUSION

The macroscopic, microscopic and physicochemical parameters revealed that the leaf powder sample was of higher degree of purity. The leaves contain covering trichomes and prismatic type of calcium oxalate crystals. It can be deduced that in order to obtain the higher yield of the extract, water may be used as the extraction solvent. The phytochemical screening revealed that *P. amboinicus* contain tannins and flavonoids as phytochemicals with antioxidant and antibacterial properties; which supports the use of the plant in traditional medicine to prevent and treat illnesses linked to free radicals and bacterial infections. The plant extract contains phytochemicals with ability to reduce Cu^{2+} and Ag^{+} to form nanoparticles. The water extract had higher antioxidant activity than the ethanol extract. The silver nanoparticle demonstrated greater antioxidant activity than the copper nanoparticle. The copper nanoparticle, water and ethanol extracts exhibited some degree of antibacterial activity on *E. coli* and *S. aureus*. The copper and silver nanoparticles have demonstrated potential antioxidant and antibacterial activities and can explored for further studies in relation to their pharmaceutical, food, and cosmetic applications.

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The authors declare no conflict of interest.

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Pharmaceutical Startups in Türkiye and Turkish Republic of Northern Cyprus (TRNC): A SWOT Analysis Inspired by the WEF Future of Jobs Report 2025

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Abstract

This study conducts a SWOT analysis of pharmaceutical startups in Türkiye and the Turkish Republic of Northern Cyprus (TRNC), informed by the World Economic Forum's Future of Jobs Report 2025. Employing a qualitative, expert-driven approach with secondary data from peer-reviewed articles, industry reports, and innovation indices (2018–2025), the research utilizes a 30-point SWOT matrix to evaluate internal strengths (e.g., Türkiye's industrial base, TRNC's EU-funded initiatives), weaknesses (e.g., regulatory hurdles, limited AI talent), external opportunities (e.g., AI-driven R&D, green manufacturing), and threats (e.g., geopolitical instability, trade barriers). Türkiye's robust ecosystem supports an offensive strategy, leveraging generic drug production, precision medicine, and digital health to compete in MENA and EU markets. Conversely, TRNC's smaller, geopolitically constrained ecosystem favors a conservative approach, focusing on niche markets like orphan drugs and clinical trial hosting through alignment with Türkiye. Strategic frameworks propose short-, medium-, and long-term priorities, including regulatory acceleration and AI talent development, to enhance competitiveness. Despite relying on secondary data and excluding the Republic of Cyprus, this study bridges entrepreneurship and global foresight, offering actionable insights for stakeholders to foster innovation and economic growth. Future research should incorporate primary stakeholder perspectives and broader regional analyses.

Keywords

Future of jobs, pharmaceutics, startups, SWOT, Türkiye, Turkish Republic of Northern Cyprus, WEF.

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INTRODUCTION

Recent research highlights the evolving landscape of entrepreneurship and innovation in the digital age. Technological advancements and globalization are reshaping business ventures, with digital transformation and global market integration significantly influencing the entrepreneurial ecosystem (Oluwadamilare et al. 2024). Success factors for start-ups in the digital era include strategic planning, team dynamics, and leveraging cutting-edge technologies like artificial intelligence (AI) and blockchain (Daraojimba et al. 2024). In the biopharmaceutical sector, AI is revolutionizing drug discovery, accelerating the development of novel therapeutics through machine learning and big data integration (Huang et al. 2024). Digital innovations are also transforming personalized medicine and adherence management, offering potential improvements in medication management quality and safety. However, challenges remain in ensuring equality among population segments and integrating these solutions into existing healthcare structures (Hein et al. 2020). The adoption of peptide therapeutics, which offer high specificity and efficacy, is another emerging trend that startups can leverage for innovative drug development (Fosgerau and Hoffmann, 2015). The global pharmaceutical market is undergoing structural transformations, driven by technological advancements and shifting economic priorities, which create both opportunities and challenges for startups (Starodubov et al. 2023). Overall, adaptability, innovation, and strategic use of technology are crucial for entrepreneurial success in the digital age.

The World Economic Forum's (WEF) Future of Jobs Report 2025 underscores the significance of biotechnology, data analytics, and personalized medicine as key drivers of future employment and economic growth. Aligning with these global trends, pharmaceutical startups in Türkiye and Turkish Republic of Northern Cyprus (TRNC) are leveraging cutting-edge technologies to innovate drug development, enhance healthcare delivery, and contribute to economic diversification.

The pharmaceutical sectors of Türkiye and TRNC have evolved significantly over the last decade, driven by a combination of regulatory reforms, innovation incentives, and growing healthcare needs. The global pharmaceutical market, which grew at a CAGR (Compound Annual Growth Rate) of 5.8% between 2018 and 2023, reached 1,607 billion dollars in 2023, growing by 8.2% compared to the previous year (Turkish Pharmaceutical Sector Report Summary, 2024). The pharmaceutical industry is experiencing steady growth, with global expenditure expected to reach US $1.5 trillion by 2023 (Gupta et al. 2020). Türkiye has implemented the world's first full pharmaceutical track and trace system, aimed

at eliminating reimbursement fraud, and preventing falsified medicines in the regulated supply chain (Parmaksiz et al. 2020). The system's success was attributed to political determination, industry incentives, reimbursement linked to verified dispensing, and flexible implementation. This system has resulted in a clean regulated supply chain, minimized fraud, and improved production quality control. However, in a study on Türkiye's pharmaceutical exports it was found out that exchange rates negatively impacted exports due to imported input costs, while industrial production index had a positive effect (Demir, 2020). External reference pricing strategies, which Türkiye employs to control drug prices, also influence market dynamics and export competitiveness (Holtorf et al. 2019). Additionally, medicine price transparency initiatives could further enhance trust and efficiency in Türkiye's pharmaceutical market, as seen in other emerging economies (Sufiza Ahmad et al. 2020).

The USA has the largest pharmaceutical market with 727.4 billion dollars and constitutes 45% of the global pharmaceutical market. The USA is followed by China with 160.8 billion dollars. Türkiye ranks 19th with a pharmaceutical market size of 10.3 billion dollars. The Turkish pharmaceutical market grew by 90.4% in local currency in 2023, while the growth recorded in dollar terms was 32.7%. Türkiye was the fastest growing market in 2023 compared to the previous year, followed by Poland with 22.9% growth rate and Mexico with 22.2% growth rate.

The global landscape of biosimilars and follow-on biologics is expanding, with 304 products identified across 18 active substance classes in various markets (Klein et al. 2022). While biosimilars offer potential for lowering healthcare costs and increasing access to biological therapies in emerging markets, challenges remain in their uptake (Chhabra et al. 2022). Government incentives play a role in promoting innovation, but their effectiveness varies across countries. In Türkiye, incentivized firms show higher innovation probability compared to non-incentivized firms, though the overall impact remains limited (Iskender and Tas, 2024). Factors influencing innovation success in emerging economies include the use of internal and external Research and Development (R&D) resources, which vary by firm type and affect internationalization strategies (Kleiner-Schäfer and Liefner, 2021). To enhance biosimilar uptake in emerging markets, implementing effective pricing and procurement policies, building provider and patient confidence, and incentivizing domestic production are crucial (Chhabra et al. 2022). The case of clopidogrel highlights how generic medicines can drive cost savings. However, investment in R&D is critical for sustained innovation (Elek et al. 2017). Asian markets, including Türkiye, are increasingly contributing to pharmaceutical

innovation, particularly in biologics and medical devices, which could inform strategies for local startups (Jakovljevic et al. 2021).

The startup ecosystem in Türkiye has been rapidly expanding, fostering innovation, job creation, and economic growth (Gencer and Sakız, 2024). The country's strategic plans emphasize entrepreneurship for social integration and competitiveness. While the startup concept is relatively new in Türkiye, the ecosystem has seen significant development, with increasing numbers of Technology Development Zones (TGBs), accelerators, and incubators (Tekin, 2021). Techno parks play a crucial role in this ecosystem, facilitating value co-creation among stakeholders (Polat et al. 2021). However, challenges such as insufficient angel investor involvement remain (Tekin, 2021). In the broader context of digital health, innovations in medical devices, pharmaceuticals, and biotechnology are advancing rapidly, offering opportunities for improved healthcare delivery and outcomes (Kasoju et al. 2023). These developments encompass smart biomedical materials, digital technologies in drug development, and applications in fields like omics biology and personalized medicine. The adoption of AI by European small and medium-sized enterprises (SMEs), including those in Türkiye, highlights the importance of digital capabilities and innovation for startup success, though external environmental factors like funding access pose barriers (Arroyabe et al. 2024). Opportunities for mutual cooperation through internationalization, particularly with Balkan countries, could further strengthen Türkiye's startup ecosystem (Tekin et al. 2021). TRNC, on the other hand, benefits from EU-funded projects like RISE and Innovate TRNC, which provide infrastructure for collaborative research and encourage public-private partnerships in pharmaceuticals and biotechnology. Moradi (2023) examines startup innovation ecosystems, identifying key actors such as universities, growth centers, and financial providers, along with critical roles like foundation, creation, and management. The study provides a framework for developing startup ecosystems, using Northern Cyprus as a case study. Ma et al. (2022) focus on university-industry collaborative R&D in the pharmaceutical sector, analyzing 30 years of publications. They highlight the importance of strategic alliances, open innovation ecosystems, and the normalization of university-industry partnerships. Both studies emphasize the significance of collaboration and knowledge exchange among various actors in fostering innovation. While Moradi (2023) offers insights for policymakers in developing startup ecosystems, Ma et al. (2022) provide strategies for future university-industry collaborations in pharmaceutical innovation.

The World Economic Forum's Future of Jobs 2025 outlines a global transition toward an innovation-driven, health-focused, and digitally enabled economy. Three trends from

the report are particularly relevant for pharmaceutical startups: Biotechnology and Genomics as Emerging Priority Sector, Increased Demand for Health-Tech Skills and Jobs, and Public-Private Partnerships and Innovation Ecosystems.

The startup ecosystem in Türkiye is rapidly expanding, fostering innovation and economic growth (Gencer and Sakız, 2024). However, startups face significant challenges, particularly in deep tech sectors where both technical and commercial hurdles exist (Arora et al. 2022). The strategic organization of innovation has evolved from specialized R&D units to C-suite involvement, emphasizing the importance of agents and capabilities in the innovation process (Cillo and Verona, 2022). In the biopharmaceutical industry, emerging technologies present both promises and challenges, necessitating new organizational models and open innovation partnerships (Boni, 2020). To address these challenges, startups must develop efficient strategies, while policymakers should create supportive environments for innovation. This includes leveraging technology parks and transfer offices to bridge the gap between research and enterprise, and implementing policies that encourage deep-tech innovation and sustainable growth in startup ecosystems (Arora et al. 2022). The adoption of Pharma 4.0 principles, which emphasize digitalization and connectivity, can further enhance startup competitiveness by improving operational efficiency and innovation capabilities. Lean production-sustainability frameworks could also guide startups in Türkiye and TRNC toward environmentally responsible manufacturing practices (Eskandari et al. 2022).

Recent studies highlight the growing importance of entrepreneurship and digital technologies in Türkiye's business landscape. The over-the-counter (OTC) drug market is expanding, with key strategies focusing on digital technologies for marketing, self-medication promotion, and health literacy improvement (Memisoglu and Bilen, 2020). However, barriers to implementing digital technologies in pharmaceutical supply chains persist in emerging economies like Türkiye (Ozbiltekin-Pala and Aracioglu, 2024). Türkiye's entrepreneurship sector shows strengths in government support and economic growth but faces challenges in boosting innovation and new technology entrepreneurship (Aydogan and Diallo, 2020).

The country's startup ecosystem is rapidly evolving, contributing to innovation, job creation, and economic strengthening. Technology parks and transfer offices play crucial roles in fostering research collaborations between universities and enterprises, although startups face high failure rates (Gencer and Sakız, 2024). A SWOT analysis of pharmacy leadership in Brazil highlights the importance of autonomy and strategic decision-making, which is relevant for

Türkiye and TRNC startups aiming to establish independent innovation ecosystems (Castro Araújo-Neto et al. 2024). These studies underscore Türkiye's efforts to embrace entrepreneurship and digital transformation across various sectors. Additionally, robust supply chain frameworks that mitigate disruptions could enhance the resilience of pharmaceutical startups in Türkiye and TRNC, particularly in volatile markets (Goswami et al. 2024).

Moreover, most prior research focuses either on large pharmaceutical companies or broader health systems, neglecting the startup layer, which is critical for fostering disruptive innovation. This gap justifies the need for a focused study that evaluates pharmaceutical startups in both countries, informed by global foresight frameworks like the WEF Future of Jobs Report. Global pharmaceutical market trends further highlight the importance of startups in driving innovation, particularly in emerging markets like Türkiye (Prozherina et al. 2021).

The primary objective of this research is to conduct a SWOT analysis of pharmaceutical startups in Türkiye and TRNC, contextualized within the global trends highlighted by the WEF Future of Jobs Report 2025. The study aims to:

1. Identify the internal strengths and weaknesses of pharmaceutical startups in Türkiye and TRNC.
2. Examine external opportunities and threats impacting these startups, including regulatory environments, market dynamics, and technological advancements.
3. Assess how these startups align with global trends in the pharmaceutical industry, particularly those related to innovation and employment as projected by the WEF.
4. Provide strategic recommendations to stakeholders for fostering a conducive environment for pharmaceutical startups in both countries.

MATERIALS AND METHODS

This research employs a qualitative, expert-driven analytical approach grounded in secondary data analysis to examine the current landscape and strategic outlook of pharmaceutical startups in Türkiye, and TRNC. The study relies on the informed perspectives of the authors—entrepreneurship researchers and strategic startup ecosystem analysts—supplemented by authoritative secondary sources, offering a structured comparison of innovation dynamics, entrepreneurial models, and future competitiveness based on global frameworks. This design allows for high-level conceptual analysis, drawing on verified secondary sources and real-world ecosystem

evaluations to identify patterns, challenges, and emerging opportunities in both territories.

All data analyzed in this study are secondary and publicly available, obtained from:

- Peer-reviewed scientific articles (Elsevier, Scopus-indexed journals)
- Industry reports (e.g., IQVIA, Deloitte Life Sciences Outlook, PwC Health Industries reports)
- Innovation and competitiveness rankings (e.g., WEF Future of Jobs Report 2025, Global Innovation Index, Global Entrepreneurship Index)
- National statistical institutions and public databases (TÜİK, Turkish Medicines and Medical Devices Agency, TRNC Ministry of Health)
- Institutional websites of pharmaceutical startups and associations
- Government innovation policies and startup incentive programs
- Public health research repositories (e.g., WHO, European Medicines Agency)

These data span 2018 to 2025, ensuring a contemporary view of post-pandemic trends, startup resilience mechanisms, and ecosystem evolution.

The research is built upon a custom-designed 30-point SWOT Analysis matrix developed by the authors to capture comprehensive strategic insights into each region's pharmaceutical startup ecosystem. The SWOT framework examines:

- Strengths: Human capital, research institutions, export infrastructure
- Weaknesses: Regulatory hurdles, capital access limitations, talent migration
- Opportunities: Biotech advancement, AI integration, green manufacturing
- Threats: Regional instability, fragmented policies, cross-border trade barriers
- WEF Future of Jobs 2025 and GEI metrics to determine sectoral skill gaps, workforce readiness, and entrepreneurship infrastructure.

The comparative scope is strictly limited to:

- Türkiye – a middle-income emerging market with a growing pharmaceutical export base and an evolving startup policy infrastructure.
- Turkish Republic of Northern Cyprus (TRNC) – a politically non-recognized region with distinct challenges and untapped potential in scientific entrepreneurship and health innovation.

Regions were chosen due to their close economic, academic, and geopolitical ties, as well as differences in policy regimes, startup maturity levels, and public-private collaboration strategies. Interpretation of the findings is based on professional judgment and academic expertise of the research team, which includes scholars, startup strategists, and innovation consultants familiar with the entrepreneurial ecosystems in Türkiye and

TRNC. The authors' reflexive analysis serves as the foundation for drawing insights and identifying cross-border synergy strategies. The process is analytical and synthetic rather than empirical. No primary data or stakeholder interviews were conducted, nor were quantitative statistical models used. The insights offered are informed evaluations anchored in current regional realities and future-oriented entrepreneurial theory. The study involves no human participants, experimental procedures, or confidential data. All data were retrieved from open-access sources or institutional databases with appropriate permissions or licenses. All sources are cited according to academic integrity principles and referenced in APA style.

RESULTS

To create a strategic framework for doing SWOT of pharmaceutics startups incorporating insights from the World Economic Forum's 2025 report, considering expert analysis, 30 key points were initially extracted as shown in table 1:

Table 1: Comprehensive table comparing the 30 key points for pharmaceutical startups across Türkiye and TRNC according to world economic forum (WEF) Future of Jobs Report year 2025.

#	Key Points	Türkiye SW	TRNC SW	Türkiye OT	TRNC OT
1	**AI and Robotics Impact**	Strengths: Established industrial base, growing interest in AI.	Strengths: Access to EU innovation frameworks.	Opportunities: Collaborations with tech-savvy EU nations.	Opportunities: Leveraging EU digital transformation initiatives.
		Weaknesses: Limited skilled AI workforce.	Weaknesses: Lack of domestic R&D capabilities.	Threats: Competition from regional leaders (e.g., UAE).	Threats: Dependence on external expertise.
2	**Automation and Augmentation**	Strengths: Emerging robotics initiatives.	Strengths: Proximity to EU automation hubs.	Opportunities: Partnering with European robotics firms.	Opportunities: EU subsidies for automation.
		Weaknesses: High initial costs for automation.	Weaknesses: High dependence on external funding.	Threats: Global automation leaders outpacing domestic growth.	Threats: Infrastructure barriers limit scalability.
3	**AI Adoption and Talent Challenges and Barriers**	Strengths: Government focus on AI, rising STEM graduates.	Strengths: Strong support from EU digital initiatives.	Opportunities: Partnering with EU tech firms, AI-driven healthcare solutions.	Opportunities: Collaborations with larger EU economies, hosting EU-funded AI research projects.
		Weaknesses: High adoption costs, shortage of local AI talent.	Weaknesses: Small market size, minimal local talent pool in AI.	Threats: Falling behind regional competitors in AI adoption.	Threats: Overdependence on external AI solutions and reliance on expatriate talent.
4	**Data-Driven Decisions and Healthcare Data Analytics**	Strengths: Emerging interest in healthcare analytics, expanding big data.	Strengths: Access to EU data-sharing frameworks.	Opportunities: Collaborating with EU data science initiatives.	Opportunities: Becoming a regional hub for healthcare data and analytics.

		Weaknesses: Data management infrastructure gaps.	Weaknesses: Minimal local expertise in data management.	Threats: Falling behind global pharma leaders in analytics-driven strategies.	Threats: Dependence on external platforms limits local innovation.
5	**Digital Access and Digital Transformation in Pharma**	Strengths: Rapidly improving pharma digital connectivity, AI-driven R&D.	Strengths: High-speed digital infrastructure, EU-supported pharma digitalization.	Opportunities: AI-driven healthcare analytics, EU digital funding programs.	Opportunities: EU-backed digital health ecosystems, positioning as an EU pharma tech hub.
		Weaknesses: Unequal access to digital health innovations, high costs.	Weaknesses: Few pharma startups integrating digital tools.	Threats: EU and UAE more advanced in digital pharma integration.	Threats: Stronger EU competitors leading in pharma digitalization.
6	**Supply Chain Digitalization**	Strengths: Strategic position as a hub between Europe, Asia, and the Middle East.	Strengths: Proximity to EU and MENA markets for cross-border logistics.	Opportunities: Partnering with EU logistics firms for blockchain-based transparency.	Opportunities: Leveraging EU-funded digital logistics programs.
		Weaknesses: Inconsistent infrastructure in rural areas.	Weaknesses: Small scale limits adoption.	Threats: Regional instability disrupting supply chain operations.	Threats: Competition from better-equipped EU logistics hubs.
7	**Quality Control Automation**	Strengths: Emerging adoption of AI in quality assurance.	Strengths: Access to EU innovation funds for automation.	Opportunities: Partnering with EU automation firms.	Opportunities: Positioning as a testing hub for EU automation projects.
		Weaknesses: High costs for automation tools.	Weaknesses: Small-scale adoption limits impact.	Threats: Falling behind global leaders in automated quality control.	Threats: High costs deter local implementation.
8	**Blockchain for Transparency**	Strengths: Government and private initiatives exploring blockchain.	Strengths: Access to EU blockchain initiatives.	Opportunities: Partnering with EU and Gulf nations to develop blockchain solutions.	Opportunities: Becoming a compliance center for blockchain-enabled pharmaceutical regulation.
		Weaknesses: Limited regulatory framework for blockchain implementation.	Weaknesses: Lack of local pharma companies using blockchain.	Threats: Regulatory uncertainties and slower adoption compared to EU markets.	Threats: EU countries implementing blockchain faster.
9	**Quantum Computing**	Strengths: Growing interest in advanced computational technologies.	Strengths: Proximity to EU-funded quantum projects.	Opportunities: Collaborating with EU quantum research hubs.	Opportunities: Hosting EU quantum experiments.
		Weaknesses: Limited R&D in quantum computing.	Weaknesses: No domestic infrastructure for quantum computing.	Threats: Lagging behind global quantum leaders.	Threats: Dependence on external research funding.
10	**Telemedicine Synergies**	Strengths: Expanding digital health startups.	Strengths: EU digital health initiatives supporting pharma.	Opportunities: Integrating AI into remote pharma consultations.	Opportunities: Becoming a regional hub for digital pharma R&D.
		Weaknesses: Regulatory uncertainty in telemedicine adoption.	Weaknesses: Few telemedicine-driven pharma firms.	Threats: UAE and EU lead in telemedicine innovations.	Threats: Reliance on EU infrastructure for telehealth expansion.

11	**Market Localization, Market-Specific Policies, and Emerging Market Focus**	Strengths: Expanding domestic pharma manufacturing sector, strategic location as pharma exporter.	Strengths: Strategic position for EU-MENA trade.	Opportunities: Exporting to MENA and Central Asia, creating startup-focused pharma incentives.	Opportunities: EU funding for small-scale pharma production.
		Weaknesses: Reliant on imported APIs, regulatory differences.	Weaknesses: Lack of large-scale pharma production.	Threats: Competition from EU-based firms, loss of startups to flexible regions.	Threats: Larger EU firms dominating pharma exports to emerging markets.
12	**AI in Regulatory Submissions**	Strengths: Investing in AI-based compliance systems.	Strengths: EU regulatory compliance framework supports AI approvals.	Opportunities: Developing AI-driven approval models.	Opportunities: Leading EU-driven AI validation projects.
		Weaknesses: Regulatory adaptation is slow.	Weaknesses: Limited domestic AI research.	Threats: EU firms already piloting AI-driven compliance systems.	Threats: Dependency on EU regulatory bodies for AI pharma advancements.
13	**Regulatory Compliance and EU Alignment Challenges**	Strengths: Proximity to EU markets, efforts to align with EU standards.	Strengths: EU membership ensures access to harmonized market.	Opportunities: Aligning local laws with EU standards to attract investors.	Opportunities: Leveraging EU pharmaceutical innovation policies.
		Weaknesses: Time-consuming compliance processes, regulatory complexity.	Weaknesses: Rigid adherence to EU frameworks limits flexibility.	Threats: Regulatory costs hampering small startups, loss of EU market access.	Threats: Non-EU competitors with more flexible regulations.
14	**Healthcare Ecosystems**	Strengths: Growing AI-driven healthcare solutions, telemedicine integration.	Strengths: EU-funded digital health infrastructure.	Opportunities: Expanding public-private partnerships to strengthen AI-driven networks.	Opportunities: Positioning as a testbed for EU-wide pharma-telemedicine integrations.
		Weaknesses: Fragmented coordination between pharma startups and hospitals.	Weaknesses: Lack of locally developed healthcare AI platforms.	Threats: EU and Gulf nations advancing faster in digital health ecosystems.	Threats: Dependence on external EU partners for technological advancements.
15	**Reskilling, Upskilling, and Skills Gap Challenges**	Strengths: Large population, growing STEM graduates.	Strengths: Access to EU-wide training resources.	Opportunities: Collaboration with online education providers, specialized training programs.	Opportunities: Attracting EU-funded reskilling projects, leveraging EU-sponsored programs.
		Weaknesses: Inconsistent training programs, mismatch with industry needs.	Weaknesses: Small-scale reskilling programs, small workforce.	Threats: Global competition for skilled workers, brain drain to EU.	Threats: Limited talent base for advanced skills, dependence on expatriate labor.
16	**Public-Private Models**	Strengths: Emerging interest in public-private healthcare funding.	Strengths: EU funding supports public-private healthcare programs.	Opportunities: Establishing partnerships with international public health bodies.	Opportunities: Collaborating with EU for pharma innovation projects.

		Weaknesses: Lack of institutional frameworks for partnerships.	Weaknesses: Small scale limits impact.	Threats: Losing out to countries with established public-private models.	Threats: Dependency on external funding for sustainability.
17	**Diverse Talent Pools and Talent Retention**	Strengths: Large youth population and a strong domestic talent pipeline in STEM fields.	Strengths: Small but diverse expatriate workforce and access to EU mobility programs for workforce expansion.	Opportunities: Promoting inclusivity to attract global talent, government-backed incentives to retain top biotech professionals.	Opportunities: EU initiatives to promote diverse hiring, EU funding for local biotech innovation hubs to keep talent engaged.
		Weaknesses: Cultural resistance to diversity in leadership roles and brain drain due to higher salaries in EU and Gulf nations.	Weaknesses: Minimal local talent pool and few high-tech pharma R&D jobs locally, leading to workforce migration.	Threats: Losing talent to more inclusive markets and to EU and UAE offering better working conditions, attracting Turkish pharma talent.	Threats: Overreliance on foreign workers and brain drain to stronger EU pharma hubs like Germany and France.
18	**Remote Work Models**	Strengths: Young population adaptable to remote models.	Strengths: Strong cross-border connections.	Opportunities: Expanding remote-friendly industries.	Opportunities: Attracting remote talent globally.
		Weaknesses: Digital infrastructure gaps.	Weaknesses: Limited domestic workforce.	Threats: Lagging behind in global remote work competitiveness.	Threats: Competition from global remote work hubs.
19	**Open Innovation and Innovation Barriers**	Strengths: Strong entrepreneurial culture and startups engaging in international R&D collaborations.	Strengths: Access to EU-backed pharma innovation networks and close proximity to innovative EU ecosystems.	Opportunities: Public-private partnerships with EU firms for co-innovation and leadership training programs to overcome resistance.	Opportunities: Hosting EU-funded pharma pilot projects and partnering with EU innovation hubs.
		Weaknesses: Government support for pharma innovation remains limited, and inertia in adopting new methods.	Weaknesses: Small local biotech ecosystem limits participation, and limited R&D funding.	Threats: Falling behind in adopting EU-standard open research models and competitors overcoming barriers faster.	Threats: Larger EU firms absorbing innovative projects before local companies benefit and reliance on external innovation sources.
20	**Targeted Drug Delivery**	Strengths: Large patient population for clinical trials.	Strengths: Strong academic collaboration with EU universities.	Opportunities: Partnering with EU biotech firms for targeted drug delivery.	Opportunities: Serving as a pilot site for EU-funded drug delivery initiatives.
		Weaknesses: Limited investment in precision medicine R&D.	Weaknesses: Lack of large-scale clinical trials.	Threats: Falling behind global competitors in personalized medicine.	Threats: Reliance on external funding limits scalability.
21	**Agility in Research**	Strengths: Growing biotech research ecosystem, increasing focus on AI-assisted drug discovery.	Strengths: EU-backed research projects support pharma innovation.	Opportunities: Faster clinical trial adoption using AI and remote monitoring.	Opportunities: Positioning as a research hub for niche pharma innovation.

		Weaknesses: Rigid regulatory approval processes delay research output.	Weaknesses: Lacks major biotech R&D institutions.	Threats: EU and U.S. firms are more agile in adapting R&D innovations.	Threats: Larger EU economies have more resources for biotech research agility.
22	**Collaborations and Partnerships**	Strengths: Strategic location enables collaborations with EU, MENA, and Asia.	Strengths: Proximity to EU partners.	Opportunities: Expanding partnerships with EU biotech firms.	Opportunities: Leveraging location for EU-MENA collaborations.
		Weaknesses: Bureaucratic challenges in cross-border partnerships.	Weaknesses: Lack of local R&D to offer reciprocal value.	Threats: Competition from regional hubs like UAE.	Threats: Overreliance on external partnerships limits growth.
23	**Diversity in Leadership**	Strengths: Rising awareness of gender diversity.	Strengths: EU-backed initiatives promote diversity.	Opportunities: Promoting inclusivity to attract international talent.	Opportunities: Partnering with EU programs to enhance inclusivity.
		Weaknesses: Slow implementation in leadership roles.	Weaknesses: Small local leadership pool.	Threats: Losing top talent to countries with inclusive leadership.	Threats: Regional competitors achieving leadership diversity faster.
24	**Customer-Centric Models**	Strengths: Large, diverse customer base allows for market segmentation.	Strengths: Small size allows personalized customer engagement.	Opportunities: Leveraging big data for customer-centric drug development.	Opportunities: Partnering with EU firms for advanced customer feedback systems.
		Weaknesses: Lack of integration of customer feedback in R&D.	Weaknesses: Limited market data for customer insights.	Threats: Competition from agile startups in the region.	Threats: Larger EU competitors dominate customer-centric models.
25	**Public Health Alignment**	Strengths: Large population provides diverse health data.	Strengths: Strong alignment with EU public health goals.	Opportunities: Collaborating with global health organizations.	Opportunities: Leveraging EU funds for health innovation.
		Weaknesses: Gaps in aligning startups with national health goals.	Weaknesses: Small population limits scalability of public health initiatives.	Threats: Limited access to public health funding.	Threats: Competition from larger EU nations for funding.
26	**Sustainability Goals**	Strengths: Growing public awareness of climate-conscious practices.	Strengths: Access to EU funding for green initiatives.	Opportunities: Tapping into EU green funding initiatives.	Opportunities: Positioning as a niche player in sustainable pharma.
		Weaknesses: Limited incentives for green pharma innovation.	Weaknesses: Infrastructure not suited for large-scale eco-friendly projects.	Threats: Global competitors achieving sustainability benchmarks faster.	Threats: Competition from countries with established green pharma capabilities.
27	**Green Transition**	Strengths: Increasing public awareness of green initiatives.	Strengths: EU funding and policies strongly support green practices.	Opportunities: Accessing EU funds for sustainability in pharma.	Opportunities: Positioning as a hub for eco-friendly small-scale pharma.
		Weaknesses: Lack of incentives for pharma startups to adopt green practices.	Weaknesses: Limited infrastructure for large-scale green production.	Threats: Global competitors advancing green transitions faster.	Threats: Higher costs of implementing green technologies.
28	**Funding Gaps**	Strengths: Expanding domestic venture capital scene.	Strengths: EU grants provide a steady funding source.	Opportunities: Attracting foreign investors.	Opportunities: Aligning with EU innovation funding programs.

		Weaknesses: Lack of government-backed funding for startups.	Weaknesses: Limited VC infrastructure.	Threats: Competition from better-funded markets.	Threats: Dependency on external financial aid.
29	**Aging Populations**	Strengths: Large workforce; potential for upskilling aging employees.	Strengths: Smaller population makes adaptation manageable.	Opportunities: Developing geriatric-focused pharmaceuticals.	Opportunities: Partnering with EU geriatric healthcare initiatives.
		Weaknesses: Rising healthcare demand for the elderly.	Weaknesses: Limited resources for elder care innovation.	Threats: Resource strain from aging population.	Threats: Growing healthcare costs.
30	**Decentralized Clinical Trials**	Strengths: Large, diverse population base for trials.	Strengths: EU ties enable participation in decentralized trials.	Opportunities: Collaborating with global pharma companies for decentralized trials.	Opportunities: Attracting EU-funded clinical research projects.
		Weaknesses: Limited access to cutting-edge trial technologies.	Weaknesses: Small population limits trial scalability.	Threats: Lagging in integrating advanced trial management tools.	Threats: Competition from larger EU countries for trial opportunities.

Strategic prioritization for pharmaceutical startups in Türkiye:

The pharmaceutical sector in Türkiye and the TRNC could be as a transformative growth, by emerging technologies, as well as evolving market dynamics, and increasing global competition. Our expert-driven analysis of 30 key factors, highlighted both opportunities and challenges for pharmaceutical startups in these regions. Türkiye benefits from its strategic geographical position, growing industrial base, and expanding digital infrastructure, yet facing challenges like limited AI talent, high automation costs, and regulatory complexities. Similarly, the TRNC, where is limited by its smaller market size and underdeveloped infrastructure, shows promise through academic interest in innovation and strong relation with Türkiye. To guide these startups toward sustainable growth and global competitiveness, we suggest a strategic prioritization framework to outline actionable short-term (1–2 years), medium-term (3–5 years), and long-term (5+ years) priorities which its addressing critical gaps and leveraging regional strengths, could be as a pivotal policy solution for both Türkiye and TRNC to become as dynamic players in the pharmaceutical landscape worldwide. The suggested framework is as shown in Table 2 for Türkiye and Table 3 for TRNC:

Table 2: Strategic Prioritization for Pharmaceutical Startups: Türkiye *(Aligned with 30 SWOT Factors)*.

Timeframe	Strategic Focus	Key Actions	Success Metrics
Short-Term (1-2 yrs)	**Regulatory Acceleration**	- Establish fast-track pathway for 5 priority drugs - Create "EU Compliance Task Force"	3 drugs approved for EU export
	AI Talent Pipeline	- Launch "Pharma AI Academy" (500 grads/yr) - 30% tax credit for AI hires	50% reduction in AI job vacancies
	Local API Production	- €10M grants for 2 domestic API plants - Mandate 20% local API use	15% drop in API imports
Medium-Term (3-5 yrs)	**Automation Leap**	- Partner with Siemens to automate 30% of manufacturing - Train 1,000 robotics technicians	25% production cost reduction
	Precision Medicine Hub	- Joint R&D with EU on 2 targeted therapies - National genomic database	1 FDA/EMA-designated orphan drug
	Green Transition	- Build 2 solar-powered GMP facilities - Adopt EU carbon-neutral certification	30% energy cost reduction
Long-Term (5+ yrs)	**Quantum Pharma**	- €50M quantum computing lab - CERN partnership for drug discovery	1 quantum-designed drug in trials
	MENA Market Dominance	- Pricing 20% below Indian generics - Distribution hubs in UAE/Nigeria	30% market share in 5 MENA countries

Table 3: Strategic Prioritization for Pharmaceutical Startups: TRNC *(Aligned with 30 SWOT Factors)*.

Timeframe	Strategic Focus	Key Actions	Success Metrics
Short-Term (1-2 yrs)	**Regulatory Alignment**	- Adopt Türkiye 's pharma regulations - Fast-track 3 generic drug approvals	2 drugs approved for Turkish market
	Digital Foundations	- Implement Turkish blockchain supply chain tools - Telemedicine integration for all startups	100% export traceability
	Talent Retention	- "Bio Visa" for Turkish Cypriot diaspora - Remote work infrastructure upgrade	20% reduction in STEM emigration
Medium-Term (3-5 yrs)	**Niche Manufacturing**	- Focus on 1-2 orphan drugs - Turkish-funded GMP facility	€50M annual export revenue
	Clinical Trial Hub	- Join Türkiye 's decentralized trial network - Specialize in geriatric trials	5 international trials hosted
	Sustainability Pilot	- Algae-based insulin production - Solar-powered labs	1 WHO-prequalified green drug
Long-Term (5+ yrs)	**Pharma-Tech Bridge**	- Regional blockchain compliance center - AI validation hub for Turkish drugs	15% of Türkiye -EU pharma audits

DISCUSSION

This study aimed to evaluate the strategic positioning of pharmaceutical startups in Türkiye and TRNC using a multi-domain SWOT analysis, inspired by the *WEF Future of Jobs Report 2025*. By focusing on emerging themes such as AI integration, regulatory agility, digital transformation, sustainability, and cross-border innovation, this research aimed to explore how these two entrepreneurial ecosystems can evolve from reactive industry players into proactive pharmaceutical innovation hubs.

The results indicate that Türkiye holds strong latent capacity for pharmaceutical innovation,

but it must strategically address regulatory bottlenecks, AI talent shortages, and API dependency to become globally competitive. On the other hand, TRNC, despite its geopolitical limitations, can carve out a niche leadership role in clinical trial hosting, sustainable pharma, and digital pharma auditing by aligning closely with Turkish systems and leveraging its compact, adaptive ecosystem.

In line with the problem statement—which centered on identifying practical innovation strategies in politically complex and economically transitional settings—this study affirms that tailored, ecosystem-specific prioritization is essential for growth. The strategic plans outlined for short, medium, and long-term implementation offer operational pathways for addressing the barriers surfaced in the SWOT analysis.

This research offers targeted insights for key stakeholders: entrepreneurs can identify high-growth opportunities in AI-driven R&D, green pharma, and orphan drugs aligned with national priorities; policymakers gain actionable, time-bound strategies with measurable indicators to guide ecosystem development; and investors or development institutions are presented with strategic entry points into the evolving Türkiye –TRNC pharmaceutical corridor, rich in cross-border IP potential and access to EU–MENA markets.

This article advances existing literature by uniquely integrating SWOT analysis with WEF megatrend insights to develop strategic roadmaps tailored for two politically distinct yet interconnected pharmaceutical ecosystems—Türkiye and the TRNC. Unlike prior studies that focus on ecosystem gaps or innovation barriers, this research offers a forward-looking, policy-grounded framework that aligns sectoral transformation with future workforce and technological trends, thereby bridging entrepreneurship, governance, and competitiveness in transition economies.

This study, while comprehensive, is subject to certain limitations: it excludes the Republic of Cyprus due to geopolitical and institutional complexities, limiting opportunities for broader regional comparisons; it relies solely on secondary data and expert opinion. Future research could expand this foundation by including a tri-partite ecosystem analysis that incorporates the Republic of Cyprus to explore cooperative competition and EU regulatory integration. It should also involve field-based validation through interviews or surveys with key stakeholders such as policymakers, startup founders, and investors. Additionally, economic impact modeling is recommended to quantify outcomes on GDP, employment, and R&D, while scenario planning under political uncertainty can help assess the resilience and adaptability of pharmaceutical ecosystems, particularly in geopolitically sensitive regions like the TRNC.

Türkiye ’s pharmaceutical startup landscape is best aligned with an offensive strategy,

leveraging its internal strengths to capitalize on growing regional and global opportunities. With a robust industrial base, a rising pool of STEM talent, strategic geographic location, and increasing government investment in AI and biotechnology, Türkiye is well-positioned to lead in areas such as generic drug production, precision medicine, and digital health innovation. The country's access to emerging markets in MENA, its partnerships with EU biotech firms, and its push toward API localization and green-certified manufacturing further reinforce its ability to pursue an aggressive growth path. These strengths enable Türkiye not only to compete with established players but to actively shape the pharmaceutical market in the Global South.

In contrast, TRNC is more suited to a conservative strategy, focusing on using external opportunities to offset its internal limitations. With a small domestic market, limited pharmaceutical infrastructure, and a high dependence on external funding and talent, TRNC lacks the internal capacity to pursue rapid or large-scale growth independently. However, through regulatory alignment with Türkiye, access to EU grants, and specialization in niche markets like orphan drugs and digital compliance tools (e.g., blockchain audits), TRNC can build a differentiated position. By leveraging its proximity to both the EU and MENA regions, and investing in telemedicine and remote-friendly infrastructure, TRNC can gradually reposition itself as a nimble and compliant bridge between larger markets.

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Investigation of Antioxidant Capacity of *Dittrichia viscosa* subsp. *angustifolia* and *Dittrichia graveolens* subsp. *graveolens*

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Abstract

This study evaluated the antioxidant properties of extracts from *Dittrichia graveolens* subsp. *graveolens* (L.) Greuter and *Dittrichia viscosa* subsp. *angustifolia* (Bég.) Greuter which were previously known as *Inula graveolens* and *Inula viscosa*. The study investigated total phenolic content, DPPH● and ABTS●+ radical scavenging tests for both ethanolic and n-hexane extracts from the plants' aerial parts.

It is shown that both *D. graveolens* subsp. *graveolens* and *D. viscosa* subsp. *angustifolia* extracts have significant antioxidant potential. This supports their traditional uses in medicine and suggests they could be valuable natural sources of antioxidants.

Keywords

Antioxidant activity, medicinal plants, natural antioxidants, radical scavenging, total phenolic content.

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INTRODUCTION

Oxidative stress, arising from an imbalance between the formation of reactive oxygen species (ROS) and antioxidant defenses, is significantly associated with the development of cancer, dementia, cardiovascular disease, and age-related illnesses. Excessive ROS can induce DNA damage, lipid peroxidation, and protein oxidation, hence facilitating cellular malfunction and disease advancement. Consequently, chemicals possessing radical-scavenging capabilities are highly regarded as preventive or adjunctive treatment agents (Valko et al. 2006).

Medicinal plants provide a significant natural source of antioxidants. Numerous plant-derived polyphenols, flavonoids, tannins, and terpenoids demonstrate significant radical-scavenging and metal-chelating properties (Sulsen et al. 2017). The Asteraceae family is one of the most pharmacologically diverse plant families. Various members are utilized in traditional medicine and contemporary phytotherapy for their anti-inflammatory, hepatoprotective, antibacterial, and antioxidant properties (Piatkowska et al. 2022).

The genus *Inula* is widely distributed throughout the Mediterranean and Middle East, comprising species rich in flavonoids, sesquiterpene lactones, and polyphenols. Numerous *Inula* species have demonstrated antioxidant, antiproliferative, and anti-inflammatory properties, indicating their potential for medicinal advancement (Tavares et al. 2019).

Inula graveolens (L.) Desf. (Synonym: *Dittrichia graveolens* (L.) Greuter) also known as camphor inula, stinkwort has been conventionally utilized as an expectorant and for the management of respiratory ailments including asthma, bronchitis, and sinusitis. Its essential oil has been utilized in aromatherapy for its mucolytic and spasmolytic attributes, and traditional practices have indicated its application for alleviating cough, inflammation, and microbiological infections. Reports also reveal the analgesic, anti-inflammatory, and antibacterial uses of this species (Seca et al. 2014).

Inula viscosa (L.) Aiton (Synonym: *Dittrichia viscosa* (L.) Greuter) is a prevalent Mediterranean herb, historically employed as an antiseptic, wound healer, and anti-inflammatory agent. The adhesive secretion has been utilized for dermatological ailments and injuries, while infusions have been used

to address gastrointestinal problems, fever, and pain. In several regions of the world, it is considered a versatile folk treatment, including accounts of its use for rheumatism, diabetes, and infections (Askın Celik et al. 2010).

Considering their ethnomedicinal origins and abundant phytochemical composition, *I. graveolens* and *I. viscosa* are attractive natural sources of antioxidant agents. This study was designed to evaluate the total phenolic content and antioxidant activity of ethanolic and hexane extracts using DPPH● and ABTS●+ radical scavenging tests.

MATERIALS AND METHODS

Reagents and chemicals

Ethanol and n-hexane were used as solvents. The study also utilized DPPH● (2,2′-diphenyl-1-picrylhydrazyl radical), ABTS+● (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), gallic acid, folin ciocalteau and NA_2CO_3.

Dittrichia graveolens and *Dittrichia viscosa* are the current accepted scientific names for the plants previously known as *Inula graveolens* and *Inula viscosa*, respectively. The genus name was changed as a result of a taxonomic reclassification.

Plant samples were collected in North Cyprus during October 2020. The plant specimens, identified as *Dittrichia graveolens* subsp. *graveolens* (L.) Greuter and *Dittrichia viscosa* subsp. *angustifolia* (Bég.) Greuter were taxonomically analyzed and confirmed by Prof. Dr. Neriman Özhatay at the Eastern Mediterranean University (EMU), Faculty of Pharmacy.

Preparation of n-hexane and ethanol extracts of *Dittrichia viscosa* and *Dittrichia graveolens* using soxhlet extraction method

Aerial parts of the 25 g of dried *Dittrichia graveolens* and *Dittrichia viscosa* was subjected to 250 mL of n-Hexane with Soxhlet apparatus. The extraction process was performed for a total of 3 hours at a temperature of 100 °C. Same procedure was followed to obtain the ethanolic extracts. Following the extraction, the n-hexane and ethanol solvents were removed using a rotary evaporator at 50°C and 0.1MPa pressure.

Total phenolic content (TPC) assay

To quantify the total phenolic content in the hexane and ethanol extracts of *D. graveolens* and *D. viscosa*. Gallic acid was used at 0.9,

0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 mg/ml concentrations as a standard.

50 µL of extracts was introduced into 96-well plates, followed by the addition of 3 mL of distilled water, 250 µL of Folin-Ciocalteu reagent, 750 µL of 20% Na_2CO_3 and 950 µL of distilled water. The extracts were incubated for 2 hours at ambient temperature for absorbance measurement. The absorbance was measured at 760 nm. Each concentration was replicated three times. For the blank solution, 50 µL of ethanol, 3 mL of distilled water, and 250 µL of Folin reagent, 750 µL of 20% Na_2CO_3 and 950 µL of distilled water.

DPPH (2,2′-diphenyl-1-picrylhydrazyl radical) scavenging assay

The antioxidant activity of both ethanolic and hexane extracts of *D. graveolens* and *D. viscosa* was assessed based on their capacity for hydrogen binding and their ability to reduce the DPPH radical, which serves as a color-change indicator of antioxidant activity.

A 0.05 mmol/L DPPH solution was prepared by dissolving 5.3 mg of DPPH in 269 mL of methanol, which was then sealed in a bottle, shaken, and wrapped in aluminium foil.

For the ethanolic extracts, samples were dissolved in methanol, and 5 µL from a series of concentrations (1.25, 1.75, 2.5, 5, 10, 20, and 30 mg/mL) were mixed with 195 µL of the prepared DPPH solution in a 96-well plate. Similarly, hexane extracts were dissolved in methanol, and 5 µL from different concentrations (10, 20, 30, 40, and 60 mg/mL) were mixed with 195 µL of the DPPH solution in a 96-well plate. Each concentration was tested in triplicate. Following a 30-minute incubation period at 25°C in a dark room, the absorbance of each sample was measured at 517 nm. A control solution was prepared by adding 5 µL of methanol to 195 µL of DPPH solution.

The DPPH scavenging capacity of the extracts was calculated using the following equation:

(% Inhibition) = [(A_control - A_sample) / A_control] x 100

A_control =The absorbance of methanol mixed with DPPH solution.

A_sample =The absorbance of ethanolic and hexane extract of *D. graveolens* and *D. viscosa* mixed with DPPH solution.

ABTS+● (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay

The antioxidant potential of the ethanolic and hexane extracts from *Dittrichia graveolens* and *Dittrichia viscosa* was measured using the ABTS+● scavenging assay. The assay is based on the reduction of the blue-green

colored ABTS+● radical, which has a high oxidizing potential, into a colorless form upon reaction with antioxidants.

Briefly, 5 µL ethanol extracts, at concentrations of 1, 3, 6, 10, and 20 mg/mL, were mixed with 145 µL of the ABTS solution in a 96-well plate. Similarly, 5 µL hexane extracts, at concentrations of 3, 6, 10, 20, and 40 mg/mL, were mixed with 145 µL of the ABTS solution in a 96-well plate. Each concentration was tested in triplicate.

Kinetic measurements were performed for 30 minutes and the absorbance values were recorded at 734 nm. A blank solution was prepared by adding 5 µL of methanol to 145 µL of the ABTS solution.

The inhibition percentage of each extract was calculated using the following formula:

(% Inhibition) = [(Blank Absorbance-Extract Absorbance) / Blank Absorbance] x 100

Blank absorbance =The absorbance of methanol mixed with ABTS solution.

Extract absorbance =The absorbance of ethanolic and hexane extracts of *D. graveolens* and *D. viscosa* mixed with ABTS solution.

RESULTS

Total phenolic content (TPC) assay

Following spectrophotometric measurement, the extracts' total phenolic content was equivalently computed using a gallic acid calibration curve. The ethanol extracts of the reference and sample plants contained nearly identical quantities of total phenolics. Total phenolic amounts of plant extracts in shown in Table 1.

Table 1: Total phenolic amounts of extracts.

Sample	Total phenol amount ($mg_{GAE}/g_{extract}$)
D. graveolens subsp. *graveolens* ethanol extract	108.8953
D. viscosa subsp. *angustifolia* ethanol extract	135.9089
D. graveolens subsp. *graveolens* hexane extract	9.068167
D. viscosa subsp. *angustifolia* hexane extract	21.2083

DPPH● (2,2′-diphenyl-1-picrylhydrazyl radical) scavenging assay

In an antioxidant dose-dependent manner, the purple color of DPPH● in the ethanol solution turns colorless. Regression equations were used to compute the IC_{50} values of the sample and standard extracts. The linearity of ethanol extracts was shown to be higher than that of hexane extracts of

both standard and sample plants, and their regression coefficient is close to 1.

The results showed that the *D. graveolens* subsp. *graveolens* hexane extract had an IC_{50} value of 23.571 mg/ml and *D. viscosa* subsp. *angustifolia* hexane extract had an IC_{50} value of 21.793 mg/ml. Based on the findings, 0.612 mg/ml was determined to be the IC_{50} value for the *D. graveolens* subsp. *graveolens* ethanol extract and the *D. viscosa* subsp. *angustifolia* ethanol extract had an IC_{50} value of 0.556 mg/ml (Table 2).

ABTS+● (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay

The ABTS+● test, which is based on the decolorization of ABTS+● solution and its absorbance inhibition at 734 nm, is a straightforward technique to examine the antioxidant activity of extracts. Consequently, better antiradical efficacy is indicated by lower absorption.

Because of its higher concentration, *D. graveolens* subsp. *graveolens* ethanol extracts demonstrated 64.65-92.26% inhibition in 30-minute kinetic tests. 2.72 mg/ml was determined to be the IC_{50} value. The ethanol extract from *D. viscosa* subsp. *angustifolia* displayed 74.62 to 85.81. 1 mg/ml was determined to be the IC_{50} value. Because of its higher concentration, *D. graveolens* subsp. *graveolens* hexane demonstrated 14.35–35.92.92% inhibition. 12 mg/ml was determined to be the IC_{50} value. The hexane extract of *D. viscosa* subsp. *angustifolia* indicated 18.64–39.63. 9,50 mg/ml was determined to be the IC_{50} value (Table 2).

Table 2: Antioxidant activity results.

Sample	DPPH results (IC_{50}) mg/ml	ABTS results (IC_{50}) mg/ml
D. graveolens subsp. *graveolens* ethanol extract	0.612	2.72
D. viscosa subsp. *angustifolia* ethanol extract	0.556	1
D. graveolens subsp. *graveolens* hexane extract	23.571	12
D. viscosa subsp. *angustifolia* hexane extract	21.793	9.5

DISCUSSION

Hexane and ethanol were used in a sequential Soxhlet extraction process to extract the herb parts of *Inula* (*Dittrichia*) *viscosa* subsp. *angustifolia* and *Inula* (*Dittrichia*) *graveolens* subsp. *graveolens* (DG) that were grown in North Cyprus. The extracts were utilized in varying quantities to evaluate the antioxidant assay using the DPPH● and ABTS+● techniques after the solvents were evaporated in vacuo. The Folin-Ciocalteau test was also used to assess the extracts' total phenol contents.

Previous studies have investigated the phytochemical profile and bioactivities of *Dittrichia* species. In *Dittrichia viscosa*, total phenolics were similar in ethanol (151.18 mg GAE/g DW) and acetone (127.09 mg GAE/g DW) extracts. Antioxidant tests (DPPH, FRAP, TAC) showed stronger activity for acetone, with lower DPPH IC_{50} (7.84 µg/mL) and higher TAC (253.52 mg AAE/g) (Mssillou et al., 2022). In another study, *Dittrichia graveolens* showed antioxidant, antimicrobial, and anticholinesterase activities. Ethanol extract had total phenolics of 86.42 mg/g and flavonoids of 117.96 mg/g. Antioxidant status was total antioxidant status (TAS) 6.933 mmol/L, total oxidant status (TOS) 12.535 µmol/L, oxidative stress index (OSI) 0.181. The extract inhibited microorganisms at 50–400 µg/mL and exhibited anti-AChE and anti-BChE activity with IC_{50} values of 25.88 µg/mL and 45.32 µg/mL, respectively (Korkmaz et al., 2023). Common phenolic compounds such as chlorogenic acid, caffeic acid, quercetin, rutin and protocatechuic acid identified in *D. viscosa* and *D. graveolens* are primarily responsible for the strong antioxidant capacity observed in both species. Their synergistic radical-scavenging, metal-chelating and reducing properties contribute significantly to the plants' overall ability to neutralize oxygen species and prevent oxidative damage (Asraoui et al. 2021; Erenler et al. 2023).

In our study, the ethanol extract exhibited markedly higher DPPH● and ABTS+● radical-scavenging activities compared to the hexane extract. The IC_{50} value for the ethanol extract was determined to be 0.612 mg/mL, whereas the hexane extract could not be reliably evaluated (IC_{50} > 20 mg/mL) due to solubility limitations. Specifically, the IC_{50} values of the *D. graveolens* subsp. *graveolens* and *D. viscosa* subsp. *angustifolia* hexane extracts were calculated as 23.571 mg/mL and 21.793 mg/mL, respectively, while the IC_{50} of the *D. viscosa* ethanol extract was 0.556 mg/mL.

Both extracts demonstrated ABTS+● radical-scavenging activity in a concentration-dependent manner. After 30 minutes of kinetic measurements, the ethanol extracts achieved 64.65–92.26% inhibition as concentration increased, whereas the hexane extracts showed only 14.35–35.92% inhibition under the same conditions.

Furthermore, the total phenolic content of the ethanol and hexane extracts was found to be 108.895 mg GAE/g extract and 9.068 mg GAE/g extract, respectively.

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The authors declare no conflict of interest.

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Importance of Plant Tissue Culture Techniques in Pharmaceutical Sciences

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Abstract

Plants serve as a significant source for the identification of novel compounds with therapeutic potential for pharmaceutical development. The rising demand for natural medicines has rendered the commercial cultivation of medicinal plants and the development of their active compounds increasingly significant. Plant tissue and cell culture techniques constitute a significant area of research in plant biotechnology, employed to improve the multiplication of medicinal plants and the synthesis of active compounds under regulated circumstances. Plant tissue and cell cultures serve as valuable alternatives in the pharmaceutical business, since they provide standardized, contaminant-free, and bio-sustainable systems for the manufacture of active medicinal ingredients. Plant tissue and cell cultures function as "bio-factories" for the synthesis of secondary metabolites, which are generally produced in minimal amounts inside plant tissues and are variably distributed across different plant organs (root, stem, leaf, fruit, etc.). Plant tissue culture facilitates the proliferation of undifferentiated plant cells, enabling the regeneration of entire plants or the cultivation of individual cells for the subsequent production of secondary metabolites. The plant tissue (explant) utilized to commence cell culture expansion at the injury site proceeds to proliferate, resulting in an unstructured cell mass termed a callus. Successful tissue culture studies that commence with callus cultures and progress to suspension cultures utilize bioreactors, enabling the quick and standardized generation of active substances. The advancement of plant cell culture research for the generation of active medicinal components and the enhancement of secondary metabolite diversity in limited quantities will yield significant contributions.

Keywords

Bioreactors, callus, plant cell cultures, phytopharmaceuticals, secondary metabolites, suspension cultures.

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INTRODUCTION

For thousands of years, therapeutic compounds have been found in nature. Numerous natural compounds with a wide range of biological activity and chemical structures have been produced by the plant kingdom, and many of these have found use in the health sciences. Of the roughly 30,000 known natural products, more than 80% come from plants. Many of the medications on the market today are straightforward synthetic versions or alterations of naturally occurring compounds. In the near future, plants will continue to produce innovative goods and chemical models for new medications. Numerous plant species that yield therapeutic herbs have had their potential medical uses assessed by scientists. The commercial significance of phytopharmaceuticals in plants has resulted in their unavoidable removal from their native habitats, endangering their survival by causing environmental and geopolitical instability. Due to careless plant collecting, a number of plants are now listed as endangered or in danger of going extinct. In order to maintain the natural habitat of plants, this has led industries and scientists to look for alternative technologies for the manufacture of phytopharmaceuticals (Sood, 2020). Research on bioreactor production and large-scale mass propagation techniques for beneficial and important metabolites in plant cell and tissue cultures has been undertaken since the late 1950s. The outcomes and endeavours resulted in multiple research projects, evaluations, and patents during the 1980s, concentrating on the industrial application of this technology. Although certain industrial uses (e.g., shikonin, taxol, ginseng biomass, berberine) and a limited number of novel products have attained semi-commercial production levels, the target level remains unachieved. The restricted commercialization results from the economic feasibility of the majority of methods reliant on plant cell and tissue cultures (Eibl and Eibl 2002). Since the Neolithic era, humans have utilized plants as natural resources, and currently, phytochemicals are predominantly employed in the medicinal, food, and textile sectors. Nonetheless, the agricultural yield of plant resources rich in phytochemicals is unachievable. Moreover, these plant species typically exhibit slow growth, possess restricted populations, and have a markedly varied and generally low concentration of target compounds. Finding a way to manufacture the desired natural products in a way that is both sustainable and financially viable presents a substantial engineering problem. Although it is

feasible to produce these compounds in a microbial or fungal host by transferring the biosynthetic pathway, this is limited because many of these pathways are complex and not fully understood (Kolewe et al. 2008). Plant cell and organ culture is a sustainable, regulated, and eco-friendly method for the industrial production of natural compounds. Moreover, improvements in cell line selection, biotransformation, product secretion, cell permeability, extraction, and scale-up have resulted in enhanced yields of plant natural products. Nonetheless, considerable challenges persist in the commercial production of high-value compounds from these sources. The recent isolation, culture, and characterization of cambial meristematic cells, together with the identification of unique routes for each active substance and their evaluation at every stage of the study, have addressed numerous possible problems. The production of phytochemicals on an industrial scale is significantly constrained, as shown in Table 1. Furthermore, PTC investigations are requisite to augment this number (Ochoa-Villarreal et al. 2016).

Secondary metabolites (SMs) constitute a diverse and often enigmatic category of molecules; although some may be mere extraneous byproducts of metabolic pathways resulting from promiscuous enzyme activity, many fulfil significant roles in defence or protection within the plant, thereby qualifying as biologically active compounds.

Plant tissue culture techniques

Plant cell, tissue, and organ culture (PCTOC) serves as a foundation for sustainable production. The techniques include callus cultures, suspension cultures, hairy root cultures, and bioreactors. Hairy root culture and other organ cultures have demonstrated efficacy in producing natural products; however, suspension culture with elicitation remains the predominant method employed to enhance secondary metabolite synthesis.

Callus cultures

Explants are the plant tissue material used to start a plant cell culture. Depending on the species, any plant tissue could be utilized as an explant with varying degrees of effectiveness. Wounding causes the cells on the explant's surface to enlarge, begin dividing, dedifferentiate, and create a mass of cells known as a callus. With the right growth media, the callus could be preserved *in vitro* indefinitely. However, in practice, determining the culture conditions and stimuli needed to exhibit this totipotency can be quite challenging and frequently solely relies on a particular plant species' responsiveness, making it a mostly empirical procedure (Barbulova et al. 2014). Culture media components and especially plant growth hormones

(regulators) significantly influence cell growth, differentiation, and metabolite production. The concentration of the medium is a critical factor in regulating callus growth and metabolite production. Establishing optimal culture conditions, encompassing both chemical and physical environments, is essential for the production of SMs from medicinal plants specific to each species. Oxidative stress significantly influences the synthesis of SMs in plants. Phenolic compounds are SMs synthesized in plants via the phenylpropanoid pathway. They serve as a defence mechanism in response to various biotic and abiotic stress conditions (Fazili et al. 2022).

Suspension cultures

Among all PTC approaches, cell suspension culture has demonstrated greater advantages than others. In suspension cultures, cells or colonies are distributed and stirred in a liquid media to enhance growth. A rapidly expanding suspension culture of single cells or tiny cell clusters is created when callus cells are cultivated in liquid media. It is now possible to scale up plant cell cultures cultivated in liquid media to much larger volumes and use them to produce commercially viable chemicals for industrial and pharmaceutical uses. Upon achieving the colony's optimal size, it is advisable to subculture the cells in a new nutritional solution. This guarantees that the cellular growth rate remains intact and that apoptosis can be averted. This is the preliminary phase in plant tissue culture (PTC) utilized for the generation of callus from a completely novel plant. To establish a suspension culture, a fragile segment of the callus is transplanted into a liquid media and maintained under optimal circumstances (such as light and temperature) to promote continuous growth and development. To get optimal yield, callus tissues are integrated and subsequently extracted from the chemical using various solvents. Scientific studies indicate that the synthesis of the desired product diminishes progressively due to significant genetic imbalance and nutrient depletion. Consequently, organ and tissue cultures are regarded as possessing greater genetic stability regarding secondary metabolite accumulation and enhanced metabolic productivity compared to suspension cultures (Isah et al. 2018). Consequently, to improve large-scale output, a thorough focus on genetic diversity, including somaclonal variation, is essential. Establishing a genetically stable cell line that produces a significant quantity of plant metabolites would constitute an efficient approach for large-scale manufacturing of these substances (Shruti and Bharadvaja, 2024).

Hairy root cultures

Hairy root cultures represent a significant advancement in the domain of plant tissue and organ culture, facilitating the enhancement of secondary metabolite production. This is accomplished through the transformation of the necessary plant species utilizing *Agrobacterium rhizogenes*, a natural vector system. *A. rhizogenes* is responsible for inducing hairy root disease in dicotyledonous plants (Giri and Narasu 2000). *A. rhizogenes*, a gram-negative soil bacterium, utilizes the machinery of dicotyledonous plants to produce its own food source by transforming its genes within the plant's genome. This transformation leads to the formation of hairy roots at the site of infection in the host plant (Shanks and Morgan 1999). Ackermann, in 1977, was the first to utilize *A. rhizogenes* for direct transformation in higher plants. The interaction between *A. rhizogenes* and plants involves a complex sequence that is also related to *A. tumefaciens*. Injured plant cells emit certain phenolic compounds, such as acetosyringone and α-hydroxy acetosyringone, which are identified by *Agrobacterium* as signaling molecules, leading to their attachment to the plant cells (chemotactic response). Upon the attachment or colonization of the bacterium to the wounded plant cells, the process results in the insertion of a T-DNA fragment from the Ti-plasmid (in the case of *A. tumefaciens*) or the Ri-plasmid (in the case of *A. rhizogenes*) into the host plant cells, leading to its integration into the plant genome. A variety of virulence genes situated within the 40-kb region of the Ti-plasmid or Ri-plasmid, known as the virulence region, play crucial roles in the process of *Agrobacterium*-mediated transformation. At the site of infection, hairy root tissues or neoplastic crown gall tumors are induced by the genes present in the T-DNA fragment. The synthesis of opines facilitates the formation of hairy roots. Bacteria utilized these opines as sources of carbon and nitrogen to infiltrate the plants (Binns and Thomashow 1988). The genes encoded by T-DNA can be expressed in infected plant cells due to the presence of eukaryotic regulatory sequences. The activation of these transformation events is contingent upon the expression of vir genes, which occurs solely in the presence of acetosyringone. Various sugars induce a high level of vir gene expression, acting in concert with acetosyringone. Root formation occurs at the location of infection. At the infection site, root development occurs due to T-DNA genes that encode for the synthesis of auxin and other rhizogenic functions. Typically, *Agrobacterium* strains possess a single type of T-DNA; however, certain Ri-plasmids, which are carriers of agropine,

include two autonomous T-DNAs known as TLDNA (left-handed T-DNA) and TR (right-handed T-DNA). The TR-DNA and TL-DNA exhibit significant similarity to the T-DNA found in the Ti-plasmid of *A. tumefaciens* and the Ri-plasmid of *A. rhizogenes* strains, respectively (Nilsson and Olsson, 1997). The processes of transformation and integration of TL-DNA and TR-DNA occur independently within the genome of the host plant. It has been established that auxin synthesis, which is encoded by the TR-DNA and TL-DNA, plays a crucial role in the production of a compound that influences the infected cells to differentiate into roots, guided by the mechanisms of endogenous auxin synthesis (Ooms et al. 1986; Shen et al. 1988). It is now recognized that the transfer of TL-DNA plays a crucial role in the onset of hairy root disease, whereas the transfer of TR-DNA does not stimulate root development from the transformed cultures (Nilsson and Olsson 1997; Sevon and Oksman-Caldentey, 2002). The capacity for transformation is influenced by various strains of *A. rhizogenes* (Giri et al. 1997; Kumar et al. 1991). It is widely recognized that hairy roots generated by different bacterial strains exhibit a range of morphologies and levels of virulence. The different variations may be characterized by the distinct plasmids present within the strain (Nguyen et al. 1992; Fazili et al. 2022).

Bioreactors

The transition from small-scale cultures to industrial-scale production requires the creation of scalable bioprocess systems (Wang et al. 2020). Cells are first cultured in shake flasks under controlled conditions to optimize medium composition and growth kinetics. Subsequently, cultures are transferred to bench-top bioreactors, which provide precise control over physical and chemical parameters, including nutrient composition, pH, temperature, oxygen supply, and shear stress (Titova et al. 2024). These systems facilitate uniform biomass growth and metabolite production, establishing the essential connection between laboratory feasibility and commercial viability. Recent advancements in bioreactor designs, such as temporary immersion systems and aeroponics-based bioreactors, have optimized nutrient delivery and gas exchange, thereby increasing metabolite yields (Ramírez-Mosqueda and Cruz-Cruz 2024).

Various types of bioreactors are employed for the scaling of SMs, depending on their dimensions and capabilities. The separation of metabolites from culture media necessitates ease of use, consistency, and a substantial level of success. Certain bioreactors are being employed in the

production of specific metabolites, taking into account factors such as culture synthesis quantity and confirmation of culture conditions. These include bubble column reactors, airlift reactors, flood reactors, and stirred reactors. Nonetheless, 'airlift bioreactors' are suitable for cells that exhibit low shear sensitivity as well as for those that are more sensitive. Airlift bioreactors are suitable for hairy and extrinsic root cultures, as well as for cells that exhibit moderate shear sensitivity, such as *Beta vulgaris* (Rudrappa et al. 2004) and alfalfa (*Medicago sativa*) (Valdiani et al. 2019). Phytoestrogens were effectively synthesized by growing hairy roots and shoots of *Genista tinctoria* L. utilizing a 'bubble bioreactor' (Łuczkiewicz and Kokotkiewicz, 2005). Microshoots of *Schisandra chinensis* (Turcz.) Baill. were cultivated in immersion bioreactors, resulting in notable growth. The cell lines of *Taxus cuspidata* Siebold & Zucc. were discovered using a 'rotating wall vessel' bioreactor, resulting in the production and optimization of Taxol concerning shear stress (Sun and Linden 1999). Slug bubble reactors have been utilized in tobacco (*Nicotiana tabacum* L.) and soybean (*Glycine max* (L.) Merr.) for isoflavone synthesis (Terrier et al. 2007). Stirred bioreactors are more appropriate for cell culture in biological processes that involve metabolite production and necessitate low shear stress. Mechanical agitation is employed to ensure sufficient oxygen supply, disrupt cell aggregates to enhance multiplication efficiency, and prevent senescence, thereby maintaining a uniform culture (Murthy et al. 2024). A stirred tank bioreactor facilitated the co-cultivation of *Streptomyces noursei* and *Aspergillus terreus*, resulting in the production of the SMs nystatin and lovastatin, respectively. A total of 50 SMs were discovered, with 19 generated by *Streptomyces noursei* and 31 by *Aspergillus terreus* exclusively. The selection of an optimal bioreactor is contingent upon the specific product, cultivation duration, and culture type. Parameters including mixing and aeration must be considered for the efficient generation of the secondary metabolite (Boruta et al. 2022; Shruti and Bharadvaja, 2024).

Strategies to increase secondary metabolite production

In research focused on improving secondary metabolite production through PTC techniques, the selection of plant species is a crucial initial step. The genotype, molecular, and biochemical characteristics of the source under investigation dictate the advancement of the study. A critical factor is the subsequent development of fast-growing, high-yield cell lines from explants derived from the mother plant.

Biotransformation

Suspension cultures can convert exogenou s chemicals into different molecules with novel properties via biotransformation mec hanisms (Fazili et al. 2022). Biotransformation is a method used to synthesize novel active ingredients with distinct qualities and to manufacture desired chemicals. Biotransformation is a process wherein primary substrates are converted into distinct substrates with novel features through the activity of specific enzymes or microorganisms. The enzymatic capacity of plant cells aids in biotransformation. These enzymes possess the ability to catalyze many processes, including regio- and stereoselective hydroxylation, oxidation-reduction, hydrogenation, glycosylation, and hydrolysis of diverse organic compounds and microorganisms. This is due to plant enzymes being regarded as essential substances for the formation of certain metabolites or other compounds with novel properties (Ishihara et al. 2003). Biotransformation differs from chemical techniques as it does not require the protection of labile functional groups (Simeo and Sinisterra 2009). For their economical large-scale production or potential use of PTCs for the biotransformation of natural chemicals, numerous cost-effective parameters have been attempted, including alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids, and amino acids.

Biotransformation occurs in various plant species, such as *Eucalyptus perriniana* F.Muell. ex Rodway, where thymol, carvacrol, and eugenol are converted into glycosides. In *Catharanthus roseus* (L.) G. Don, glycosylation biotransformation of capsaicin and 8-nordihydrocapsaicin occurs in cell cultures (Shimoda et al. 2007). Biotransformation in the cell suspension cultures of *Catharanthus roseus* and *Platycodon grandiflorus* (Jacq.) A.DC. results in the synthesis of a novel chemical, 1b-hydroxyl desacetylcinobufagin, along with additional unidentified compounds exhibiting cytotoxic properties against HL-60 cell lines (Ye et al. 2003). In tobacco, hyoscyamine is transformed into scopolamine through biotransformation (Moyano et al. 2007). The immobilized cell technology has been employed in the generation of SMs. The immobilized cell technology has been utilized in the generation of SMs (Fazili et al. 2022).

Elicitor usage

The term "elicitor" denotes a chemical or physical substance that contributes to defense mechanisms by promoting the accumulation of secondary metabolites in plant cells or tissues in response to invading pathogens (Ramachandra Rao and Ravishankar, 2002). Elicitors are classified into three categories based on their nature:

biotic, abiotic, and exogenous or endogenous. Biotic elicitors are derived from plants, pathogens, or other biological sources. They possess defined structures and specific chemical compositions (Shruti and Bharadvaja, 2024). Biotic elicitors can have complex compositions, such as yeast cell walls, mycelial cell walls, fungal spores, or a defined composition. These can include carbohydrates (alginate, pectin, chitosan, guar gum, mannuronate, guluronate, mannan, galacturonides), proteins (cellulase, oligandrin), or lipids (lipopolysaccharides). The most common abiotic elicitors are: methyl jasmonate, salicylic acid, calcium chloride, sodium alginate, and metal ions (copper sulphate, silver nitrate, vanadium sulphate). Abiotic elicitors can be divided into three categories: chemical, hormonal, and physical. Elicitation has been used extensively in plant cell and organ cultures to increase the production of secondary metabolites. Depending on the particular secondary metabolites, different biotic and abiotic elicitors have an impact on secondary metabolite production in plant tissue cultures. The use of elicitors as agents in plant tissue culture systems offers enormous potential for the large-scale generation of secondary metabolites. Naik and Al–Khayri (2016) studied on abiotic and biotic elicitor's role in secondary metabolites production through *in vitro* culture of medicinal plants.

Immobilization

Immobilization is a technique that maintains cells within an appropriate matrix support while allowing the movement of substrates and products, thereby improving cell viability in the stationary phase and potentially facilitating prolonged biomass production, ultimately lowering the cost of plant-derived SMs (Isah et al. 2018). The generation of capsaicin from immobilized cells of *Capsicum* sp. demonstrates a 100-fold increase in foam and gel, exemplifying the significant influence of cell immobilization on secondary metabolite production. Calcium alginate was utilized in MS medium containing $CaCl_2$ for the immobilization of *Plumbago rosea* L. in the production of plumbagin, a crucial therapeutic compound. Compared to control, uncrosslinked alginate, and $CaCl_2$-treated cells, immobilization in calcium alginate enhanced plumbagin synthesis by three, two, and one-fold, respectively (Vanisree et al. 2004; Shruti and Bharadvaja, 2024).

Permeabilization

Permeabilizing agents, such as dimethylsulfoxide (DMSO), chitosan (a polysaccharide), and isopropanol, have been employed to improve the accessibility of enzymes. Hairy roots of *Datura*

stramonium were treated with Tween 20 through permeabilization, resulting in the retention of 25% of hyoscyamine, sufficient to maintain cell viability (Khelifi et al. 2011). Triton X-100 and n-hexadecane were utilized in the production of ajmalicine in *Catharanthus roseus*. The hairy root cultures were permeabilized with these agents, leading to a 98% increase in ajmalicine production. The observed 12-fold and 16-fold increases in metabolites with DMSO and Triton X-100, respectively, indicate the potential of this application for scaled-up production. Additional methods of permeabilization, such as ultrasonication, ionophoretic release, and electroporation, have been employed for the release of SMs.

All operate based on the principles of low current, ultrahigh pressure, and high electric field pulses. Valepotriate production in *Valeriana glechomifolia* F.G.Mey. increased significantly when subjected to ultrasonication for approximately 5 minutes, without impacting biomass production (Vaessen et al. 2019; Shruti and Bharadvaja, 2024).

Precursor feeding

This strategy involves the addition of intermediates during the initial stage to enhance metabolite production. Precursors are substances that transform SMs through a biosynthetic pathway. The final productivity of the compound is enhanced by reacting with the intermediate involved in any biosynthetic pathway. *Catharanthus roseus* suspension cultures exhibited increased production of secologanin and indole alkaloids upon the addition of loganin, tryptophan, and tryptamine. *Taxus cuspidata* phenylalanine enhanced paclitaxel accumulation. The anthocyanin content in the L-phenylalanine repetitive feeding culture was 30% and 81% greater than that in a single L-phenylalanine-fed culture and a non-fed culture, respectively (Cerezo et al. 2020). A study demonstrated that the administration of four precursors (L-phenylalanine, cinnamic acid, ferulic acid, and sinapic acid) in *Larrea divaricata* Cav. tissue cultures enhanced the production of phenolic compounds to varying extents. L-Alanine used in *Plumbago indica* for the biosynthesis of Plumbago resulted in a 14-fold increase in production when added on the 14th day of cultivation (Jaisi and Panichayupakaranant, 2017). The duration and concentration of the precursor must be considered during its incorporation into the culture medium. Failure to do so will inhibit secondary metabolism through a feedback mechanism (Murthy et al. 2014).

Metabolic engineering

Metabolic engineering systematically produces desirable products using various molecular biology techniques that modify specific biochemical reactions.

In metabolic engineering, the target pathway is overexpressed, rate-limiting steps are circumvented, the catabolism of the target product is inhibited, competing pathways are obstructed, or any practical combination of these strategies is employed to improve production (Oksman-Caldentey and Inzé, 2004). Metabolic engineering employs diverse strategies to enhance the efficiency and productivity of SMs by inhibiting competitive pathways. This inhibition elevates the metabolism of intermediates involved in biosynthetic pathways, facilitating the discovery of novel molecules with enhanced therapeutic potential (Verpoorte et al. 2002). The conversion of pulegone to menthofuran resulted in the blockage of the monoterpene network, facilitating increased production of menthol. Similar to the inhibition of biosynthetic pathways, the accumulation of SMs can be attained (Allen et al. 2004). In *Catharanthus roseus*, the upregulation of ORCA3 led to an increased expression of genes associated with the terpenoid indole alkaloid pathways, resulting in a threefold increase in alkaloid production (Van Der Fits and Memelink, 2000). To enhance the production of flavonoids and apocarotenoids, the DET1 regulatory gene was silenced using RNA interference (Davuluri et al. 2005). This strategy is employed for the production of various molecules; however, limitations within metabolic pathways remain a significant barrier. This can be addressed by integrating genomic expression data with metabolome profiling to enhance understanding of the regulation of SMs during biosynthesis. For example, the integration of proteomics with elicitation strategies resulted in increased production of *Maytenus ilicifolia* Mart. ex Reissek cell cultures (Paz et al. 2017).

Genetic engineering

The capacity *of in vitro* culture to modify genes in plants has facilitated the application of genetic engineering for the production of a diverse array of metabolites with enhanced therapeutic value. Especially beneficial when there are challenges in isolating particular plant compounds. In recent years, biotechnological applications have been utilized for the production of SMs. MicroRNA (miRNA) significantly regulates gene expression in eukaryotes; thus, this strategy has been employed in phytochemical synthesis.

For instance, in *Papaver somniferum*, the synthesis of zylisoquinoline alkaloids (BIA) was monitored through miRNAs. Their role in the biosynthesis of SMs has been addressed in Gupta et al. (2017). Another strategy is virus-induced gene silencing (VIGS), in which the gene of interest is targeted using the genome of the virus.

The gene for ornithine decarboxylase (ODC) in *Nicotiana tabacum* was silenced. Consequently, the addition of methyl jasmonate to hairy roots resulted in a decrease in nicotine and anatobine levels. This silencing approach enabled the analysis of the specific roles of six genes in *Papaver somniferum*, a task not accomplished through various biochemical studies. Genome editing technologies, including clustered regularly interspaced short palindromic repeats with protein 9 endonuclease (CRISPR/Cas9), zinc finger nucleases (ZFNs), and transcription activator-like endonucleases (TALENs), have been applied in plants to modify specific traits of crop species. A novel compound, benzylisoquinoline, was isolated using the CRISPR/spCas9 type II approach, resulting in a reduction of morphine and thebaine content (Belhaj et al. 2015; Shruti and Bharadvaja, 2024). To comprehend the necessity of plant-derived SMs, it is essential to examine their feasibility at an industrial scale. Due to their inconsistent production, reduced growth rates, low oxygen requirements, and heightened sensitivity, processing plant cultures is challenging.

Advantages of plant cell, tissue and organ culture techniques

Humans have recognized the application of plants and plant extracts in traditional medicine since antiquity. Over time, humans have developed a thorough understanding of beneficial plants through continuous interaction with nature. Plants are sessile organisms, rendering them incapable of evading biotic and abiotic stressors throughout their life cycle. This challenge has prompted the evolution of a wide range of structurally and functionally diverse metabolites to mitigate these stressors and adapt to dynamic environments. The increasing global population and rising demand for food, pharmaceuticals, and industrially relevant natural products highlight the critical necessity for sustainable biotechnological solutions. Plant biotechnology, specifically plant cell, tissue, and organ culture (PCTOC), offers a controlled, renewable, and scalable platform for the extraction of metabolites to satisfy this demand (Chandran et al. 2020; Ozyigit et al. 2023). PCTOC facilitates the *in vitro* cultivation of plant cells, tissues, and organs, thereby enabling the production of high-value phytochemicals without dependence on seasonal or geographical limitations. The metabolic complexity of plant systems has historically constrained our capacity to optimize metabolite production through traditional empirical methods (Alamgir, 2018; Wijerathna-Yapa et al. 2025).

Because of the chemical diversity of natural products obtained from plants and the rising desire for natural medications and

supplements, medicinal plants are very important to the pharmaceutical industry. However, locating pharmaceutically active natural compounds is frequently difficult because of the slow growth of some species, low yields observed in nature, and unpredictable variability in accumulation. Although there are a number of production options, such as natural harvesting, total chemical synthesis, semi-synthesis from isolated precursors, and expression of plant pathways in microbial systems, low natural yields, chemical complexity, and ignorance of the entire biosynthetic pathway preclude many of these options for some medicinal natural products, which makes PTC technology an alluring sourcing alternative. PTC is appropriate for metabolic engineering, environmental optimization, and scale-up. SMs constitute a diverse and often enigmatic category of molecules; although some may be mere extraneous byproducts of metabolic pathways resulting from promiscuous enzyme activity, many fulfill significant roles in defense or protection within the plant, thereby qualifying as biologically active compounds (Kolewe et al. 2008).

The extraction of SMs from plants presents several challenges, including environmental factors, political and labor instabilities in producing countries, uncontrollable variations in crop quality, the inability of authorities to prevent crop adulteration, and losses incurred during storage and handling (Smetanska, 2008).

PTC methods were originally investigated for the commercial manufacture of several high-value SMs, facilitating sustainable manufacturing that circumvents the low yields of natural harvesting and the elevated costs of intricate chemical synthesis. Despite achieving some commercial success, there are process restrictions related to low product yields and intrinsic manufacturing unpredictability. Diverse techniques, including subtraction, *in situ* product removal, and metabolic engineering utilising individual genes and transcription factors, are being devised to address these constraints. The PTC manufacturing platform has recently been augmented to encompass pharmaceutically active heterologous proteins. Plant systems are advantageous because they can generate complex proteins that are appropriately glycosylated, folded, and assembled, without the contamination risks from toxins associated with mammalian or microbial production systems. Additionally, PTC isolates transgenic material from environmental influences, offers more controlled conditions than field-grown crops, and facilitates protein release into the medium, hence decreasing subsequent purifying expenses (Leal et al. 2018; Wilson and Roberts, 2012).

Organic substances known as SMs do not directly contribute to an organism's typical growth, development, or reproduction. In contrast to primary metabolites, the absence of SMs does not cause immediate death; instead, it impairs the organism's ability to survive, fecundity, or appearance over time, or it may not alter anything at all. SMs frequently have a significant impact on interspecies defenses and plant defenses against diseases, pests, and herbivores. SMs may have a defensive role through toxicity, deterrence/anti-feedant activity, or serving as building blocks for physical defense mechanisms. In general, SMs are used by humans as flavorings and medications (Bennett and Wallsgrove, 1994; Sood, 2020).

Secondary metabolism has attracted a lot of attention lately because of the growing commercial significance of SMs, especially the potential to modify the synthesis of bioactive plant metabolites through tissue culture technologies. To investigate and produce plant SMs, PTC technologies for plants were offered as a potential tool near the end of the 1960s. The development of PTC technology has made it possible to manufacture a wide variety of SMs. Compared to conventional plant breeding techniques, the production of plant metabolites in cell cultures has a number of benefits. First, regardless of changes in soil or environment, PTC techniques enable the controlled synthesis of advantageous chemicals. It is simple to multiply plant cells to generate particular metabolites in any environment, whether tropical or alpine. The absence of bacteria and insects in cultivated cells is an additional benefit. Additionally, PTCs' precise modulation of metabolite processes and automated control of cell growth lower labor costs while boosting production. It is possible to collect organic matter from callus cultures and create metabolites year-round in a controlled setting by choosing genotypes that produce more SMs as mother plants. (Vanisree et al. 2004; Ravishankar and Ramachandra Rao, 2000; Sood, 2020).

Commercially interesting plants are increasingly being grown on an industrial scale due to the high demand for natural compounds from a world population that is constantly expanding. However, in many cases, cropping and extraction techniques have not been optimized, and as a result, the entire process has proven to be economically unsustainable. Since PTCs consistently provide standardized, contaminant-free, and bio-sustainable products whose production can be readily expanded to an industrial scale, they undoubtedly constitute a viable alternative for the manufacturing of pharmaceutical active ingredients (Table 1).

Table 1 lists various industrially produced secondary metabolites by PTC techniques

along with their market prices, natural sources, economic uses, biological activities, and current pricing which is adapted from Malik et al. (2016).

Large strides have been achieved in "plant tissue culturing" techniques, which have taught how to handle and manipulate plant cells in the laboratory under sterile and controlled settings, thanks to more recent developments in the study of plant physiology and cell biology. Plasticity and totipotency are the two traits that set plants apart from all other living things and are essential to comprehending PTC. The ability of any plant part or plant cell to develop into a complete organism or differentiate into any of its cells or tissues is called totipotency. (Condic, 2014). The ability of plants to modify their metabolism to adjust their growth and development to the environmental conditions around them is known as plasticity. Plant cells naturally have the ability to divide from nearly any tissue, regenerate, and activate several developmental and metabolic pathways in response to stress. Through a process of cell dedifferentiation and subsequent differentiation, *in vitro* plant cells exhibit extremely high degrees of flexibility and can regenerate tissues, organs, and even entire plants. Furthermore, given the right conditions, a certain quantity of undifferentiated totipotent cells is always preserved in the meristems found inside the vascular system or at the terminals of shoots and roots, enabling rapid cell division (Lee et al. 2010; Barbulova et al. 2014).

The synthesis of shikonin, the inaugural secondary metabolite manufactured on an industrial scale, was successfully amplified at Mitsui Petrochemical Corporation in Japan *via* a two-stage bioreactor method. Bioreactors with a capacity of 200 L for upstream processes and 750 L for downstream shikonin production yielded 23% of shikonin and its derivatives after 9 and 14 days of incubation, culminating in a total duration of 23 days. The efficacy of the PTC approach is evident when comparing the shikonin yield from bioreactor cells to the 2% yield achieved from wild plants over a span of 3 to 7 years. The production of *L. erythrorhizon* cell cultures was 60 mg per gram of cells per week, which is 1000 times greater than the production of plant roots, necessitating a duration of 7 years. Each bioreactor operation yielded around 5 kg of shikonin, aiding Mitsui Chemicals in achieving an annual production of 65 kg out of its 150 kg demand (Tabata and Fujita, 1985; Malik et al. 2016). The significance of employing the PTC approach is evident, given that a 100 mg skikonin now costs 3200 € (Table 1).

Table 1: Commercial-scale produced SMs by PTC techniques are used as pharmaceuticals, and their sources, biological activities, and prices.

Secondary Metabolite	Plant species (source of SMs)	Family of plant species	Biological activity	Industrial price (US$/Kg)	Value*, ** (€/ 100 mg)
Ajmaline	*Rauwolfia serpentina*	Apocynaceae	Sedative	75000	38**
Ajmaline	*Catharanthus roseus*	Apocynaceae	Anticancer	37 000	38**
Artemisinin	*Artemisia annua*	Asteraceae	Anti-malarial	400	140*
Berberine	*Coptis japonica*	Ranunculaceae	Antiseptic	3250	260*
Codeine	*Papaver somniferum*	Papaveraceae	Sedative	17000	144*
Colchicine	*Colchicum speciosum*	Colchicaceae	Antitumoral	35000	24.3*
Digoxin	*Digitalis lanata*	Scrophulariaceae	Cardiatonic	3000	36,9*
Diosgenin	*Dioscorea deltoidea*	Dioscoreaceae	anticancer, infertility treatment, diabetes	1000	244*
Ellipticine	*Orchrosia elliptica*	Apocynaceae	Antitumoral	240 000	1110*
Ginsenosides	*Panax ginseng*	Araliaceae	Adaptogenic, dietary supplement, Immunomodulator	15000	540*
Morphine	*Papaver somniferum*	Papaveraceae	Analgesic	340000	252*
Paclitaxel	*Taxus canadensis*	Taxaceae	Antitumoral	20400	2112*
Podophyllotoxin	*Podophyllum peltatum, Linum sp.*	Berberidaceae	Anticancer, antiviral, antihelminthic, cathartic, vesicant	800000	141*
Sanguinarine	*Sanguinaria canadensis*	Papaveraceae	Antibiotic, expectorant, analgesic	4800	2560*
Shikonin derivatives	*Lithospermum erythrorhizon, Arnebia sp.*	Boraginaceae	anticancer, anti-bacterial, anti-HIV, cosmetics, natural dye	4500	3200*
Quinine	*Cinchona ledgeriana*	Rubiaceae	Anti-malarial	500	506*
Vinblastine	*Catharanthus roseus*	Apocynaceae	Anticancer	1 000 000	1858*
Vincristine	*Catharanthus roseus*	Apocynaceae	Anti-leukemic	3000 000	2500*

*,**Based on data from Sigma-Aldrich* and TargetMol** the economical value is expressed in Euro (€) per 100 mg; the cost varies according to purity (2025/09).

CONCLUSION

The majority of medications on the market today are synthetic versions of naturally occurring plant compounds. Due to the fact that natural medicines have fewer adverse effects, many people are turning to them. PTCs offer interesting possibilities for the production of high-value SMs without the necessity of whole plants (Shruti and Bharadvaja, 2024).

PCTOC consists of a collection of *in vitro* methodologies for the aseptic propagation and modification of plant cells, tissues, and organs within regulated environmental settings. PCTOC functions as the fundamental biotechnological platform from which the scalable and reproducible production of plant-derived metabolites commences. It provides a sterile, controllable, and reproducible environment that eliminates the unpredictability linked to field-based cultivation. This ensures consistent metabolite biosynthesis irrespective of seasonal, regional, and environmental variations. This method also assists in protecting endangered species collected from the wild for their active compounds, ensuring a sustainable approach to metabolite production (Wijerathna-Yapa et al. 2025).

In vitro tissue culture is a viable method for producing high-value, structurally complex natural products, particularly when the mother plant is an overfished, slow-growing, or low-yielding plant. Similar benefits can be obtained by producing pharmaceuticals using plant culture systems, such as cost savings, speed, low human pathogen burden, and scalability; these benefits are specific to plant products and rely on production efficiencies in comparison to those provided by other sources.

The mechanisms via which plants synthesize secondary metabolites are now easily managed and regulated. Methodologies such as plant cell, tissue, and organ cultures can enhance the synthesis of pharmacologically significant secondary metabolites. Understanding the SM pathway in economically significant plants has led to the predominant use of *in vitro* approaches. Future innovations may enable the effective extraction of significant and unknown compounds from medicinal plants, offering renewable sources for essential pharmaceutical ingredients.

Specifically, advancements in plant cell, tissue systems, and organ cultures provide valuable techniques for the cultivation of herbal SMs. Suspension culture and elicitation are effective methodologies in cell culture for augmenting the generation of SMs. Research indicates that hairy root

and shoot cultures are more reliable than cell cultures for the production of therapeutically significant SMs. These approaches are advantageous because they autonomously respond to seasonal and environmental variations. Genetic engineering is employed to enhance the mass synthesis of important SMs for the regulation of cellular pathways. This knowledge and comprehension of cellular pathways in both plants and their cultures are utilized as information pertaining to cellular and molecular levels. To address the issues of low yield and scale-up, it is essential to better the understanding of biosynthetic pathways and employ contemporary biological approaches, such as elicitors and permeabilizing agents, to improve both the efficacy and production of the resultant chemicals. Moreover, the application of innovative technologies like metabolomics and transcriptomics has yielded insights into the regulatory mechanisms and biosynthetic routes of SMs, which can be utilized to formulate new strategies for augmenting the production of SMs. Furthermore, the employment of PTCs and bioreactors can offer a regulated and effective framework for the large-scale production of SMs. The ongoing advancement and use of innovative techniques and technologies enable us to fully exploit the potential of SMs for the pharmaceutical industry (Shruti and Bharadvaja, 2024). Diverse procedures, including as nutrient optimization, ambient condition enhancement, application of stress-inducing chemicals, and selection of high-yield strains, have been utilized to augment production. Recent advancements in functional genomics and metabolite profiling provide unparalleled opportunities to use the biochemical potential of plants for the production and design of new molecules (Shruti and Bharadvaja, 2024).

With the application of cutting-edge technologies like gene editing and environmental factor manipulation, tissue culture should realize its full potential within the near future.

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