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Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)
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Sincerely,
Prof. Dr. Nazım ŞEKEROĞLU
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Anticancer activity of *Amaranthus spinosus* L. (Tanduliya): A Review

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Abstract

Cancer is served typical life-threatening disease with common risk factors. Developing therapeutic measures for cancers has aroused attention for a long time. However, the problems with conventional treatments are in challenge, including side effects, economic burdens, and patient compliance. It is essential to secure safe and efficient therapeutic methods to overcome these issues. The current review portrays the functions of *Amaranthus spinosus* L. (Tanduliya), and its phytoconstituents against several types of cancers, and explores the possibility of developing these agents as a promising candidate for cancer treatment. *A. spinosus* is an edible plant, belonging to the family Amaranthaceae widely found in Asian countries like India, Sri Lanka, Japan, and Indonesia, and used for dietary and medicinal values. Among the various beneficial pharmacological effects of *A. spinosus*, anticancer activity is presumably less studied. *A. spinosus* contains several secondary metabolites like glycosides, phenolic compounds, steroids, terpenoids, saponin, carotenoids, tannins, etc. that strongly assure their anticancer activity. The effects of *A. spinosus* and its various derived phytoconstituents have been shown to anticancer activity against breast, hepatocellular, prostate, and colorectal cancer in various preclinical models. The in-depth review of existing studies has shown the promising anticancer activity of *A. spinosus* extract, and its bioactive molecules by inhibiting the different stages of cancer, including initiation, promotion, and progression. Besides valuable nutraceuticals, *A. spinosus* has multi-targeted actions like antioxidant, anti-inflammatory, immunomodulatory activity and the nontoxic nature of *A. spinosus* probably plays a crucial role in killing cancerous cells.

Key Words: Amaranthaceae, Amaranthus, Anti-inflammatory, Antioxidant, Cancer, Immunomodulatory

1. Introduction

The efficacy of anticancer therapeutics is limited by agent-specific adverse effects. Apart from impacting prognosis, acute or persisting therapy-induced normal tissue damage substantially impacts the patient´s quality of life (Temel et al., 2010). Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body (Doll and Peto, 1981). Multiple treatments are utilized for the cure of cancer like surgery with chemotherapy and/or radiation therapy which produced various side effects like anemia, nausea, vomiting, weight loss, infection, diarrhea, edema, fatigue, etc. Estimated national

expenditures for cancer care in the United States in 2018 were $150.8 billion (Siegel, 2022). In future years, costs are likely to increase as the population ages and more people have cancer. Costs are also likely to increase as new, and often more expensive, treatments are adopted as standards of care.

Natural products play an important role in cancer prevention and treatment. Phenolic compounds from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting the cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways (Huang et al., 2009; Sharma 2021).

Amaranthus spinosus L. species are widely distributed and cultivated in Asia, Africa, America, Australia, and Europe. Leaves and succulent stems of Amaranthus are inexpensive and excellent sources of protein with essential amino acids lysine and methionine, carotenoids, ascorbic acid, dietary fiber, and essential minerals, such as calcium, magnesium, potassium, phosphorus, iron, zinc, copper, and manganese (Sarker et al., 2019). *A. spinosus* belongs to the family Amaranthaceae and is a tropical and subtropical annual and perennial herb with a purple or greenish stem that grows up to 100-130 cm tall and is extensively found in India, Africa, Southeast Asia, and the United States of America. Branches and grooves of the spiny herb are widely distributed on erect glabrous herbs. Oval or elliptic-lanceolate leaves have an attenuate base and measure 3-8 x 2-4 cm. The petiole is up to 4 cm long, and the apex is obtuse or subacute. Flowers are found in axillary, sessile clusters, or terminal panicled spikes. Ovate-lanceolate bracts and bracteoles are small (Figure 1) (Kirtikar and Basu, 2001). *A. spinosus* leaves are used in traditional African medicine for skin disorders, gastrointestinal disorders, and inflammatory and painful conditions. *A. spinosus* is used to treat children's ophthalmitis, convulsions including nutritional deficiencies, in many parts of Africa (Ibewuike et al., 1997). The leaves of *A. spinosus* are used by the tribal people of Kerala, Sikkim, and Manipur in India to treat piles, leprosy, diabetes, high blood pressure, and rheumatic pain in addition to gastric disorders (including indigestion and peptic ulcer). The plant's decoction is described as being used to stop miscarriages in ancient Indian texts. To induce bowel movements in children, boil leaves and roots are used (Basu et al., 2019; The Wealth of India, 2006; Kirtikar and Basu, 2001).

Just a few previous reviews offer detailed knowledge of the progress of this significant research area. One of the previous review articles presents a brief overview of the compilation and exploration of the therapeutic properties of *A. spinosus* with various pharmacological activities like antioxidant, antidiabetic, immunomodulatory, antiprotozoal, anti-malarial, hepatoprotective, antigenic, and allergenic activity, etc. (Jhade et al., 2011). There are no publications on the overview of *A. spinosus* extract and its phytoconstituents in cancer. In this review, we discuss the possible health advantages of *A. spinosus* as well as the method by which it inhibits cancer progression. This review, in detail, portrays the functions of *A. spinosus* and its bioactive constituents against several types of cancers and explores the possibility of developing these agents as anticancer pharmaceuticals.

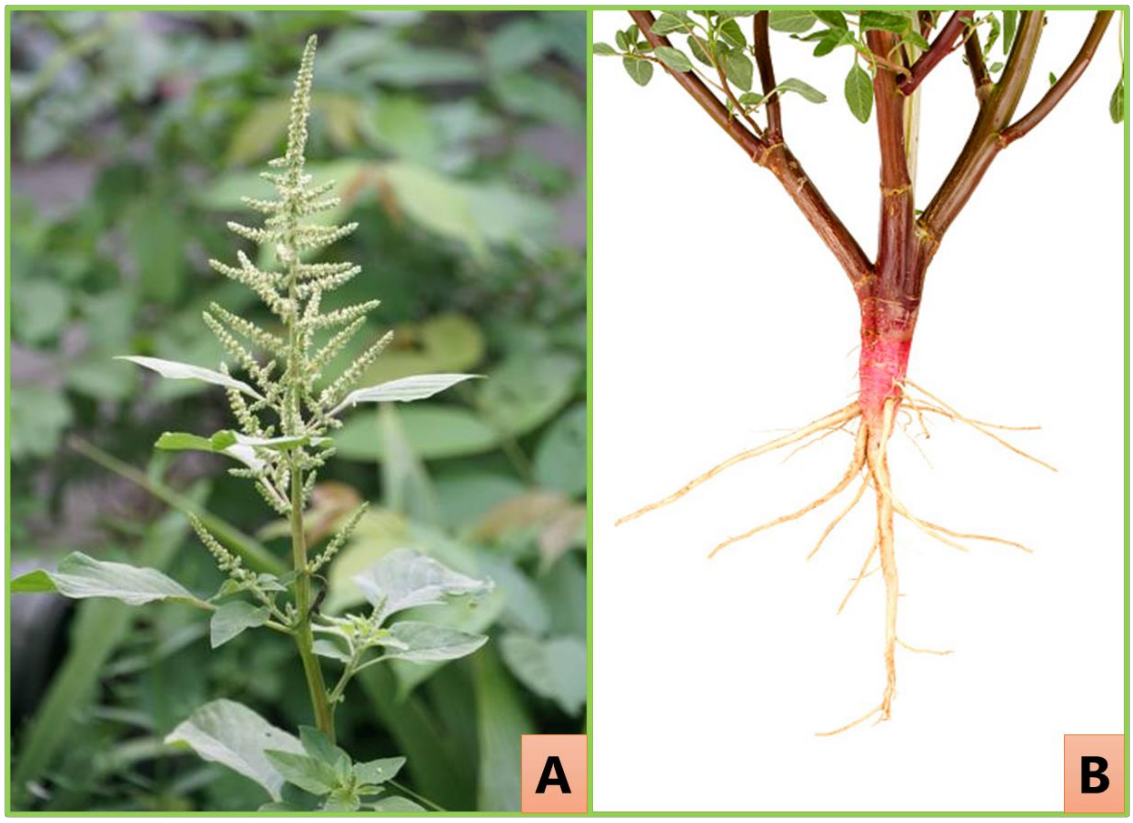

Figure 1. Photographs of *Amaranthus spinosus* L. (A) Natural habitat (B) Root.

Material and Methods

We thoroughly investigated Google Scholar and PubMed using relevant keywords like "*Amaranthus spinosus*," "Tanduliya," "Chaulai," "Prickly Amaranth", "Spiny amaranth", "Spiny pigweed", "Thorny amaranth," "Traditional use," "Ethnopharmacology," "Ethnobotany," "Phytoconstituents," "Chemical constituents," "Anticancer," "Anti-inflammatory," "Antioxidant", "Immunomodulatory" "cytotoxic" "Apoptosis" and "Toxicological study." Pertinent peer-reviewed research articles were retrieved from databases like Web of Science, Scopus, Embase, and MEDLINE. The articles were chosen for inclusion in the current review after all of the articles underwent authenticity, dependability, and relevance evaluations. ChemDraw Ultra 15.0 was used to illustrate the structures of all the phytoconstituents after they had all been verified using PubChem.

3. Results and Discussion

3.1. Nutraceutical properties

In a comparative study, the calcium content in dry leaves of *A. spinosus* was found to have a higher value (4500 mg/100 g dry weight) as compared to other species of Amaranthus (*A. tricolor*, *A. viridis*, and *A. blitum*) (Srivastava, 2011). As a leafy vegetable, weedy *A. spinosus* has remarkable protein, dietary fiber, carbohydrates, calcium, potassium, magnesium, phosphorus, sulphur, iron, manganese, copper, zinc, sodium, chlorophylls, β-cyanins, β-xanthin's, betalains, β-carotene, vitamin C, phenols, and flavonoids, etc. *A. spinosus* genotype WAS13 have high nutritional and antioxidant activity because it contains highest nutrients, pigments, vitamins, phenolics, flavonoids, and antioxidant (Figure 2, Table 1) (Sarker et al., 2019).

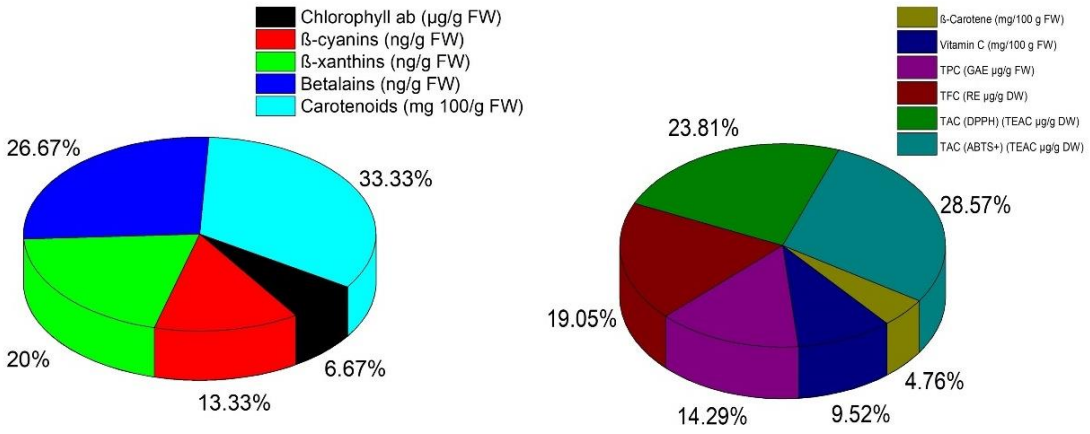

Figure 2. Nutraceutical value present in *A. spinosus*

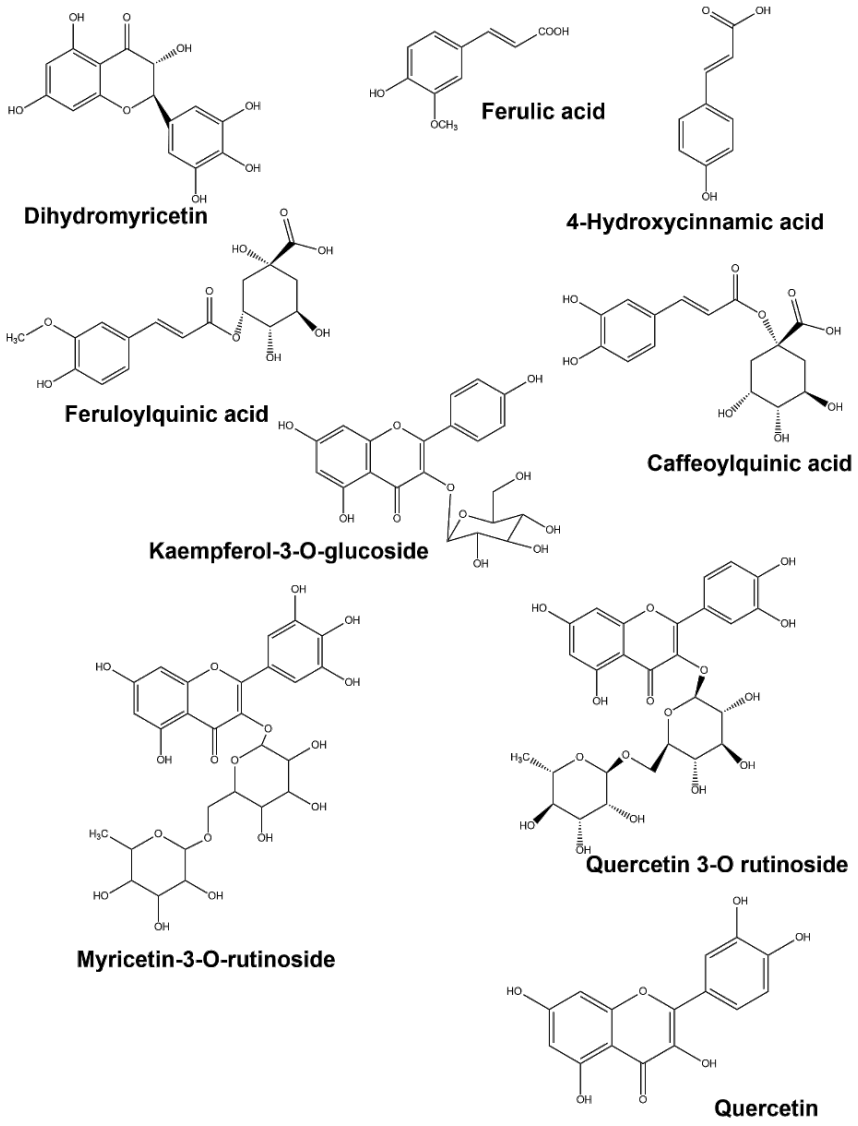

Figure 3. Chemical structures of major phytoconstituents isolated from *A. spinosus*

Table 1. Nutraceutical's composition of *A. spinosus*

Nutrients	Amount
Moisture (g/100 g)	84.47±1.34
Protein (g/100 g)	5.78±0.06
Fat (g/100 g)	0.63±0.02
Carbohydrates (g/100 g)	4.41±0.03
Energy (Kcal)	27.89±0.46
Ash (g/100 g)	5.18±0.04
Fiber (g/100 g FW)	11.24±0.72
K (mg/g FW)	6.48±0.04
Ca (mg/g FW)	2.44±0.05
Mg (mg/g FW)	3.02±0.05
P (mg/g FW)	0.68±0.005
S (mg/g FW)	1.25±0.04
Fe (µg/g FW)	15.34±0.09
Mn (µg g^{-1} FW)	10.23±0.06
Cu (µg/g FW)	2.04±0.07
Zn (µg/g FW)	10.99±0.13
Na (µg/g FW)	25.73±0.14
B (µg/g FW)	7.25±0.06
Mo (µg/g FW)	0.32±0.02

3.2. Phytochemistry

In an exclusive study, authors have reported alkaloids, glycosides, terpenes, and sugars as the major phytochemicals in the roots of *A. spinosus* (Jhade et al., 2011). Preliminary phytochemical screening of the 50% ethanolic leaves extract of *A. spinosus* showed the presence of carbohydrates, phenolic compounds, phytosterol, alkaloids, and flavonoids (Mishra et al., 2011). The hexane, chloroform, ethanolic and aqueous extract of aerial parts of *A. spinosus* showed the presence of saponin, carbohydrate, tannin, protein, glycoside, flavonoid, and phenol (Khanal et al., 2015). The total polyphenol content in the *A. spinosus* aerial methanolic extract was 194.21± 9.22 mg GAE/g extract, and the total flavonoid content in the extract was estimated to be 18.68± 3.67 mg QE/g. The LC-MS analysis showed the presence of various polyphenols in aerial methanolic *A. spinosus* extract like dihydromyricetin, ferulic acid, 4-hydroxycinnamic acid, feruloylquinic acid, kaempferol-3-O-glucoside, caffeoylquinic acid, myricetin-3-O-rutinoside, quercetin-3-O-rutinoside and quercetin (House et al., 2020) (Figure 3). Mondal et al. isolated (14E,18E,22E,26E)-methyl nonacosa-14,18,22,26 tetraenoate fatty acid from the whole plant of chloroform fraction of *A. spinosus* (Mondal et al., 2016).

3.3. Antioxidant activity

Free radicals are extremely reactive chemicals that can damage cells. They develop naturally in the body and are vital to many regular cellular processes (Diplock et al., 1998). Free radicals can damage DNA, proteins, and cell membranes when they are present in high concentrations, which is harmful to the body. Free radical damage to cells, particularly DNA damage, may lead to the formation of cancer and other illnesses (Valko et al., 2007). Chemicals known as antioxidants interact with and deactivate free radicals to stop them from harming. Most of the endogenous antioxidants that the body uses to combat free radicals are produced by the body. However, the body may obtain the remaining antioxidants it requires from exogenous sources, mainly the diet. Dietary antioxidants are a common name for these exogenous antioxidants and are abundant in fruits, vegetables, and grains. Vitamins A, C, and E (α-tocopherol) and β-carotene are a few examples of dietary antioxidants (Davis et al., 2012). Increased exogenous antioxidant levels inhibit the kinds of damage caused by free radicals that have been linked to the development of cancer *in vivo* studies.

The aerial methanolic extract of *A. spinosus* showed antioxidant activity by ferric reducing antioxidant power activity, scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and hydrogen peroxide (H_2O_2) activity (IC_{50}=24.38±1.44 mg/mL, 63.94± 3.72, and 28.55± 1.87 respectively) (House et al., 2020). Similarly, ethyl acetate leaves extract of *A. spinosus* possesses good radical scavenging activity (IC_{50} =53.68 µg/ml) (Bulbul et al., 2011). Leaves extracts of *A. spinosus* with and without vitamin C reverse the increased malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) levels in rats (Faponle et al., 2015).

The antioxidant capacity of *A. spinosus* extracts significantly reduced the amount of oxidative free radicals in rats and aided in rapid wound healing (Paswan et al., 2020). The methanolic seed extract of *A. spinosus* demonstrated concentration-dependent scavenging activity against DPPH and H_2O_2. Methanol seed extract had the highest radical scavenging activity value at 450 mg/mL (84.13%), but it was less effective than vitamin C (94.21%) (Rjeibi et al., 2016).

In addition, *A. spinosus* methanolic leaves extract showed antioxidant activity against DPPH radicals, nitric oxide radicals, and butylated hydroxyl anisole (Kumar et al., 2010). *A. spinosus* root extracts scavenged DPPH radicals in a dose-dependent manner (Barku et al., 2013). The oral administration of *A. spinosus* increases the hepatic antioxidants GSH, SOD, CAT, and glutathione peroxidase (GPx) levels (Prince et al. 2021).

The bioactive antioxidants potential of the major categories of phenolic acids and flavonoids derived from chalcone and cinnamate-dependent pathways of phenylpropanoid metabolism showed antioxidant properties of different plant parts of *A. spinosus* L. (Kar and Bhattacharjee, 2022).

3.4. Analgesic and Anti-inflammatory activity

Inflammation-generated oxidative stress and damage to macromolecules are prominent physiological features of all chronic diseases. Angiogenesis, tissue remodeling, stimulation of neoplastic transformation, and metastasis are all known to be induced by chronic inflammation and oxidative stress, in addition to being associated with tumor cell proliferation and growth (He et al., 2017; Grimm et al.,2013; De Oliveira et al., 2017).

When such normal tissue homeostasis is disrupted, cellular proliferation, as well as programmed cell death, becomes imbalanced, which leads to the development of a malignant state (apoptosis). It is known that activating the caspase cascade through the death receptors pathway or the mitochondrial death signaling, whose components have either been proapoptotic (Bax, Bad) or antiapoptotic (Bcl-2, Bcl-XL), can cause apoptosis (Huang and He, 2011; Yang et al., 2009; Laulier and Lopez, 2012). In order to combat cancer, one method used in the development of anti-cancer drugs is to cause programmed cell death in cancer cells (Ginwala et al., 2009). In particular, traditional uses of plants have drawn attention as plant-based foods, as their regular consumption may be linked to a lower cancer incidence (Aravindaram et al., 2010; Cerella et al., 2010; Ravishankar et al., 2013).

The ethyl acetate extract of *A. spinosus* leaves managed to prevent the compound 48/80 secretagogue's ability to induce systemic anaphylactic shock in an animal model. It inhibited the release of histamine, mast cell degranulation, and membrane disruption, suggesting a potential role in the avoidance and treatment of anaphylactic reactions (Patil et al., 2012). The whole plant of methanolic extract of *A. spinosus* had significant central and peripheral anti-nociceptive potency as well as anti-

inflammatory activity (Baral, 2010; Olajide et al., 2004). Similarly, the extracts reduced inflammation-related pain, improved hot plate reaction times, and significantly increased tail immersion test results, all of which support further research into the clinical application of the extracts in conditions related to pain (Taiab et al., 2011). The methanolic aerial extract of *A. spinosus* inhibited lipoxygenase and scavenged nitric oxide (House et al., 2020). Leaves extracts of *A. spinosus* have demonstrated significant membrane stability, which improves anti-inflammatory responses (Rajasekaran et al., 2014). Research on castor oil-induced diarrhea and gastric mucosal integrity suggests that this plant extract's activity is likely mediated by the inhibition of prostaglandin biosynthesis (Olumayokun et al., 2004). A significant dose-dependent peripheral analgesic activity was demonstrated by *A. spinosus* methanolic extract. It significantly lessened the abdominal contractions brought on by acetic acid (Senthil Kumar et al., 2010). Methanolic extract of *A. spinosus* whole part exhibited significant anti-nociceptive and anti-inflammatory action in a mouse model. The extracts improved reaction times on a hot plate, decreased pain brought on by inflammation, and displayed a significant amount of activity in a tail immersion test (Md et al., 2011). The alcoholic extract and whole plant of *A. spinosus* possess both anti-inflammatory and analgesic activity in the carrageenan-induced paw edema model (Bharti et al., 2022; Jamaluddin et al., 2011).

3.5. Immunomodulatory activity

Previous studies have shown that tumor-bearing animals and cancer patients can both exhibit immunosuppressive effects, indicating that the immune system plays a significant role in immune surveillance against cancer cells (Chen et al., 2012). Therefore, it has been recognized that increasing the host's immune response may be a way to prevent tumor growth without endangering the host (Jiao et al., 2008). The immune system serves as the body's main line of defense against cancer because it can identify and eliminate developing tumors. Female BALB/c mouse spleen cells were significantly stimulated to proliferate by *A. spinosus* water extract. Studies conducted in vitro revealed that *A. spinosus* aqueous extracts had immunostimulant activity by directly promoting T cell proliferation and subsequent B lymphocyte activation in a dose-dependent manner (Lin et al., 2005). The immune-modulating effects of *A. spinosus* water extract on naturally occurring and dexamethasone (DEX)-induced apoptosis in murine primary splenocytes. The results demonstrated that the alcoholic extract prevented splenocyte apoptosis both naturally occurring and induced by DEX (Linn et al., 2008). The humoral and cell-mediated immune responses were both significantly increased by the alcoholic extract, but they were both significantly decreased by the petroleum ether extract (Tatiya et al., 2007).

3.6. Cytotoxicity and Anticancer activity

Despite advancements in technology and medicine over time, cancer remains a global concern (Seyed et al., 2016). It is the second major cause of death worldwide and accounts for thousands of fatalities annually. Despite the fact that the main reasons for chemotherapy failure continue to be target mutation, toxicity, and resistance, chemotherapy is still a vital treatment option for various cancers (Tiwari et al., 2016). Therefore, more study is needed to identify and create novel anti-cancer drugs that can resolve these chemotherapeutic failure causes.

In this context, the MTT assay demonstrated that the fatty acid significantly and dose-dependently inhibited cell proliferation of HepG2 cells (IC_{50}=25.52 µmol/L). This antiproliferative outcome was superior to linoleic acid (IC_{50} =38.65 µmol/L), but comparable to that of the standard drug, doxorubicin (IC_{50}= 24.68 µmol/L). The

G2/M transition was arrested as a result of the novel fatty acid induction of apoptosis, which was mediated by the downregulation of cyclin B1, the upregulation of Bax, and the downregulation of B-cell lymphoma 2 (Bcl-2) (Mondal et al., 2006). The most common type of liver cancer, hepatocellular carcinoma, accounts for the majority of cancer deaths worldwide (Parkin et al., 2002). The fatty acid (14E,18E,22E,26E)-methyl nonacosa-14,18,22,26 tetraenoate) isolated from *A. spinosus* showed significant antiproliferative activity that was mediated by the induction of apoptosis in HepG2 cells. The second most frequent form of cancer in men is prostate cancer. Scientists are working hard to identify potential sources in the commonly consumed foods that prevent the growth of prostate cancer. Ethanolic extract of *A. spinosus* was tested for its cytotoxic and anticancer properties using lymph node carcinoma of the prostate (LNCaP) cell lines and the brine shrimp lethality assay. The findings indicated the cytotoxic activity of *A. spinosus* against Artemia salina (LC_{50}= 920 µg/mL). However, at the 400 µg/mL tested concentration of *A. spinosus* failed to inhibit cell proliferation (Octaviani et al., 2013).

Apoptosis is a physiological process that is characterized by DNA fragmentation, membrane blebbing, chromatin condensation, and cell shrinkage (Sharma et al. 2007). Apoptosis is a crucial regulator of tissue homeostasis, and an imbalance between cell death and proliferation can lead to the development of tumors (Fulda and Debatin 2003). The water extract of *A. spinosus* exhibited apoptosis induction and cytotoxic activities in *Allium cepa* L. root meristematic cells and human erythrocytes (Prajitha and Thoppil, 2013). The standard crude extract showed cytotoxic activity against HeLa cancer cell lines. It was found that crude extract showed a significant effect on the estimated cell counts of apoptotic death of HeLa and HeLa-R cells (Sharma et al., 2020). The hydroalcoholic extract of *A. spinosus* roots extract showed a non-cytotoxic effect (LC_{50} = 1.178 mg/mL). Almost twelve percent of those with confirmed cases of breast cancer later develop metastatic illness, or breast cancer which has moved from the breast to other regions of the body, despite advancements in detection of breast cancer, diagnosis, and treatment (Peart, 2017). Methanolic aerial extract of *A. spinosus* showed cytotoxicity (IC_{50}=64.90± 3.91 mg/mL) against the breast cancer cell line (MCF-7). While the aerial methanolic extract displayed greater antiproliferative potential (IC_{50}=82.11± 7.02 mg/mL) in triple-negative breast cancer cell lines (MDA-MB-231) (House et al., 2020). In breast, colorectal (HT-29), liver (HEPG2), and normal cell lines, the methanol extracts of *A. spinosus* leaves significantly inhibited the growth of tumors. The amount of tumor growth and the number of viable cells is significantly reduced by the methanol leaves extract (Rajasekaran et al., 2014; Joshua et al., 2010). *A. spinosus* leaf methanol extract exhibited chemo-protective action in rats by reducing oxidative stress and inflammation in response to doxorubicin-induced multi-organ damage (Akinloye et al., 2023).

3.7. Toxicity studies

Acute oral toxicity testing with *A. spinosus* leaves extract at the highest dose (2000 mg/kg) did not reveal any animal lethality or behavioral changes (Kumar et al., 2010). A single oral dose of the extract (5000 mg/kg/b.w.) did not result in any fatalities or toxicological symptoms in a gross necropsy. Repeated doses of the extract (500 and 1000 mg/kg, b.w.) did not result in any deaths or notable changes in body weight or the relative weight of the vital organs in sub-chronic oral toxicity. Additionally, there was no discernible change in the hematological, biochemical, or histopathological parameters. Blood glucose levels were found to reduce, but this did not result in organ damage, demonstrating the plant's safety (Atchou et al., 2021).

4. Limitations and Future Perspectives

The nutrient-rich food *A. spinosus* may offer enormous opportunities for the community who suffer from nutrient deficiency to overcome hidden hunger and achieve nutritional sufficiency. The different pharmacological properties of this plant could be attributed to its bioactive compounds and nutritional value. *A. spinosus* pharmacological activity was greatly aided by the antioxidant activity of its pigments, β-carotene, vitamin C, phenolics, and flavonoids. The current study indicates that *A. spinosus* has undergone less pharmacological research, but it is a great source of phenolics, flavonoids, and antioxidants that have a wide range of pharmacological and therapeutic effects. Therefore, more research on the bioactive components of *A. spinosus* is required to assess its therapeutic efficacy in animal models and clinical studies to verify its anticancer activities at the cellular and molecular level, and also ensure its status as a functional food.

5. Conclusion

A. spinosus has been used for its dietary and therapeutic benefits. It is a perennial herb used to treat and prevent a number of ailments including cancer, inflammation, pain. *A. spinosus* bioactive substances demonstrated significant anticarcinogenic effects through a number of mechanisms, including cancer cell apoptosis, cell cycle arrest, and activation of the angiogenic cascade. Bioactive phytochemicals from *A. spinosus* inhibit cell growth, apoptosis evasion, adhesion, invasion, migration, and metastasis in a diverse array of preclinical models of breast, hepatocellular, prostate, and colorectal cancer.

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Author Contribution

GKT conceptualized and designed the study, while the first draft of the manuscript was collaboratively written by GKT and MH. MH provided critical revisions to the work. All authors thoroughly reviewed and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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HOST AND PLANT TAXONOMY AS INFLUENTIAL FACTORS IN CONTROLING *IN VITRO* SPECIFIC CELL-MEDIATED RESPONSE IN HERBIVORES

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Abstract

Objective: This study monitored several alcoholic vegetal extracts from the adjuvant and immune cell stimulating perspective in farmed herbivores, differing by their digestive physiology.
Material and Methods: Blood from randomly selected farmed ruminants (Romanian Spotted dairy cows, n=28 and Angora goats, n=19) and Romanian draft horses (n=27) was used to measure the *in vitro* effects on specific cell-mediated reactivity of alcoholic extracts of *Calendula officinalis* L., *Echinacea angustifolia* D.C. and *E. purpurea* (L.) Moench by the *in vitro* whole blood blast transformation test, in 96 well plates, after 60 to 72h of incubation. Cell growth was quantified by an orto-toluidine technique. Student's t- test was used to evaluate the statistical significance of the differences.
Results and Discussion: The *in vitro* growth indices were lowest in goats (*C. officinalis* – 58.52±10.02%, *E.angustifolia* – 50.06±11.67%, *E.purpurea* -50.79±10.98%) and higher in bovine (*C. officinalis* – 69.9±2.65%, *E.angustifolia* – 74.9±10.1%, $p<0.05$), and increased towards *E. angustifolia* versus *C. officinalis*.
In vitro responses to *C. officinalis*, *E. angustifolia and E. purpurea* were the most pronounced in horses and similar for all of these extracts.
Conclusion: All the extracts showed inhibiting effects in bovine and goats, but not in horses, supporting host-based differences. The biological activity of the tested extracts was plant species dependent.

Key Words: Ruminants, horses, plant extracts, blast transformation

1. Introduction

Antibiotic resistance represents in the actual world one of the biggest threats to human and animals health and food security, while it is a naturally occurring phaenomenon but the process is being enhanced by misuse of antibiotics in human and veterinary medicine (WHO, 2023). To prevent further aggravation of the antibiotic resistance, natural solutions are sought for, such as organic acids, feed enzymes, and pro- or pre-biotics to control microbes. Plant extracts represent a historical, inexhaustable, valuable and available tool to control health and welfare in pets and food animals as well (Windisch, 2008). The immune modulating properties demonstrated in case of several extracts derived from medicinal plants are

the subject of extended research as part of a global trend (Amirghofran et al., 2000; Barbour et al., 2004; Jiménez-Medina et al., 2006Amirghofran et al., 2009; Chand et al., 2011; Bhatt et al., 2014).

Essential oils from various plants positively influence the local gut immunity in non-ruminants (Zeng et al., 2015) while others such as noni (Nanak and Mengi, 2010), purple coneflower (Fors, 2015) or holy basil (Mukherjee et al., 2005) extracts increased the lymphocyte activation in people, horses and ruminants respectively. Some researches indicated that in different orders of carnivores the immunological activity of plants depended on species and also differed within the same plant family (*Compositae*) (Spinu et al., 2016). The results of such studies are valuable for the field of veterinary medicine given the negative impact of several factors related to inadequate husbandry and welfare on the immune system functionality in farmed animals (Disler et al., 2014). To our knowledge, there are no comparative studies on the combined influence of plant and animal taxonomic ranking on immune effects of plant extracts.

This study aimed to compare three alcoholic extracts from plants belonging to the same family (*Compositae*), but different genera (*Calendula, Echinacea*) and species (*E. purpurea* and *E. angustifolia*) for their ability to enhance the adaptive cell-mediated immunity in farmed herbivores, differing by their digestive physiology, as it is in ruminants (bovine, goats) and monogastric, non-ruminants (equine).

2. Material and Methods

2.1. Biological material

Randomly selected semi-intensively farmed ruminants (Romanian Spotted dairy cows, n=28 and Angora goats, n=19) and extensively farmed Romanian draft horses (n=27) were used to monitor the potential influence exerted by their differentiated immune morphology and physiology in eliciting responses to active principles from different vegetal extracts. Peripheral blood of the experimental animals obtained from the jugular vein, sampled on heparine (50 IU/ml), was used to investigate the potential of alcoholic vegetal extracts to improve leukocyte reactivity. The blood was transported to the laboratory under isotermal conditions (37°C) and processed in maximum 4 h after sampling.

2.2. Leukocyte blast transformation test

The *in vitro* leukocyte blast transformation test provides evidence on the potential of monocytes and lymophocytes to further react *in vivo* to sensitizing antigens. Commercial alcoholic extracts of *Calendula officinalis, Echinaceea purpureea and Echinaceea angustifolia* (Plantextract, Romania) for human use, produced according to the German Homeophatic Pharmacopeia, were used to *in vitro* treat the whole blood cultures.

One ml of each blood sample was diluted with four times the amount of RPMI 1640 (Sigma-Aldrich, USA) supplemented with 5% FCS (Sigma-Aldrich, USA) and antibiotics penicillin (1000 IU/ml, streptomycin 1000 µg/ml)(Sigma Aldrich, USA), at pH 7.4; when needed the pH was corrected with a sterile 2% sodium bicarbonate solution. Each blood+supplemented culture medium mixture was placed in 96-sterile-well plates (200 µl per well), in duplicate for each extract/species (Khokhlova et al., 2004). Six *in vitro* experimental variants were tested for each individual animal, namely (1) untreated control culture, (2) phytohaemagglutinin-M (PHA)(1µ per well), (3) 70° alcohol and (4–6) alcoholic vegetal extracts of *Calendula officinalis*, *Echinacea purpurea* and *Echinaceea angustifolia* (1.5 µl/well). The most effective *in vitro* concentrations of the extracts were established during preliminary studies by the same technique. Subsequent to an incubation at 37.5°C in a 5% CO_2 atmosphere for 60 h for equine cultures and

72 h for ruminant blood samples, glucose consumption was evaluated by an orto-toluidine colorimetric method with a subsequent spectophotometrical reading at 610 nm wavelength (SUMAL PE2, Karl Zeiss, Jena, Germany), using the reagent as a blank [17,18] . The stimulation/inhibition index (S/I) was calculated as: S/I %=[(IG–GR)/MG]x100, where S/I =blast transformation index, IG= the initial glucose concentration in the supplemented RPMI 1640 and GR=glucose residue in the sample after incubation (Spinu et al., 2016).

2.3. Statistical analyses

Average values and standard error were calculated by use of Excel program. Student's t test was applied to evaluate the statistical significance of the differences which program?

3. Results and Discussion

Traditional medicine worldwide uses numerous herbs and medicinal plants to prevent or treat most variable range of human and/or animal diseases. Observation of behavioral processes even indicated use of medicinal plants in nature by certain species of animals (Page et al., 1992).
The *Compositae* (*Asteracea*) family includes about 10% of all flowering plant species, consisting of over 1,900 genera and 32,000 species within the order *Asterales* with a vaste palet of forms and geographical distribution (Mandel et al., 2016, Marshall et al., 2023).

The uses of different *Asteraceae* are highly variable, with extremes as for cooking oils (*Helianthus annuus*, common sunflower) but also horticultural importance (*Calendula officinalis* marigold, *Echinacea* spp. (coneflowers). One important role of numerous members of this family are in herbal medicine use for human health (Jennifer et al., 2020). Similar to their high variety, there is a broad range of phytochemical compounds, including polyphenols, phenolic acids, flavonoids, acetylenes and triterpenes in the plants of *Asteracea*, responsible for their pharmacological effects (Hueza et al., 2019). Numerous plant compound are immunologically active such as flavonoids, polysaccharides, lactones, alkaloids, diterpenoids and glycosides (Jantan et al., 2015).

3.1. Dynamics of the leukocyte subpopulations

The trend of the *in vitro* leukocyte response to plant extracts was the most steady in equine (Fig. 1). The differences in responses to extracts from *Calendula* and *Echinacea* genera as well as to the different species of the same genus (*Echinacea*) indicated differences in composition and/or concentration of the active principles and also a different perception of the active plant principles by the immune system of each animal species.

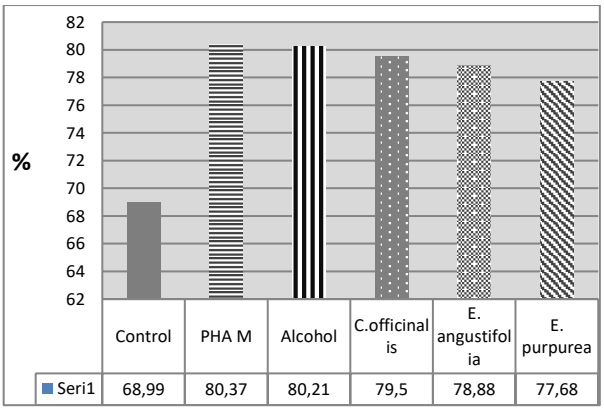

Fig. 1. Blast transformation indices in horse leukocytes treated with alcoholic vegetal extracts

In vitro responses to *C. officinalis*, *E. angustifolia and E. purpurea* were the most pronounced in horses (Fig. 1) and very close for all extracts: 79.50±6.39%, 78.88±3.74 and 77.68±5.93%, respectively.
The *in vitro* blast transformation indices were the lowest in goats (*C. officinalis* - 58.52±10.02%, *E. angustifolia* - 50.06± 11.67%, *E. purpurea* - 50.79±10.98%)(Fig 2).

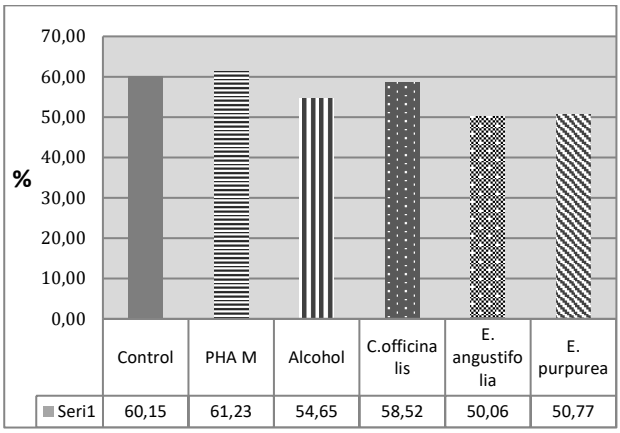

Fig. 2. Blast transformation indices in Angora goat leukocytes treated with alcoholic vegetal extracts (average) The decimal points in figure should be point not comma

In bovine (Fig. 3), the values were higher for all tested extracts (*C. officinalis* – 69.9±2.65%, *E.angustifolia* - 74.9±10.1%, *E. purpurea* -73.22±9.95% $p<0.05$ when compared to goats) and increased towards *E. angustifolia* versus *C. officinalis*.

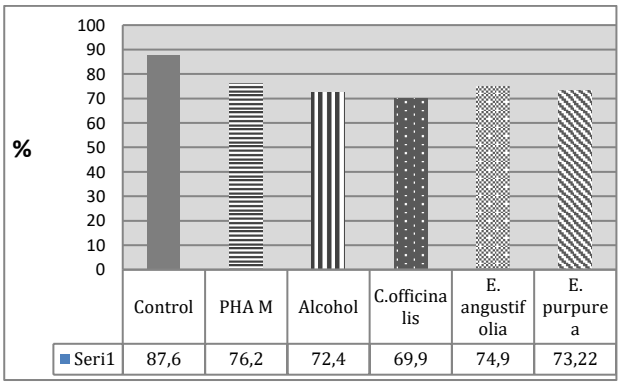

Fig. 3. Blast transformation indices in bovine leukocytes treated with alcoholic vegetal extracts (average)

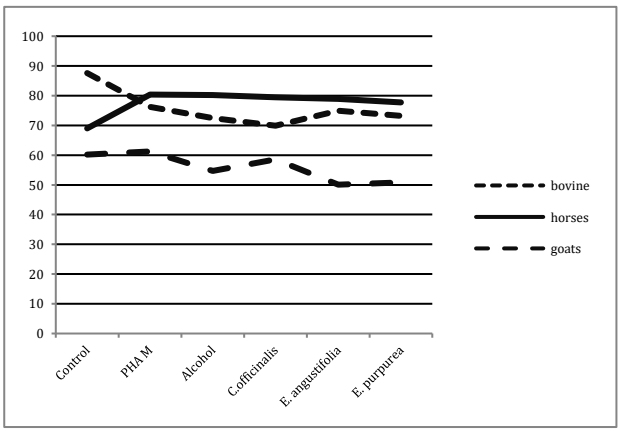

Fig. 4. Leukocyte blastogenic development trend in tested herbivorous species (average)

In this trial, there were few statistically significant differences between the blast transformation indices recorded for the three herbivorous species. When comparing goats with both bovine and horses, the cell-mediated response to both *Echinacea* species were statistically relevantly lower ($p<0.05$). Nevertheless, although some of the components of the tested plants are similar (polysaccharides, chlorogenic acid, caffeic acid) there are some differences in their chemical composition, even if belonging to the same genus (echinacoside is only present in *E. angustifolia* and absent in *E. purpurea*, while cynarin is typical for *E. angustifolia*) (Barnes et al., 2005). An aqueous extract of *C. officinalis* was mentioned to activate the lymphocyte and produce antitumor activity, mantioning the global composition (polysaccharides, proteins, fatty acids, carotenoids, flavonoids, triterpenoids and saponins) of the product (Jimenez-Medina et al., 2006). Although the *Echinacea* species were cited to influence both innate (Sun et al., 1999) and adaptive cell-meditede immunity (Zhai et al., 2007), *E. purpurea* was found less stimulating T_H cells than *E. angustifolia*.

The present study investigated the effects of the two *Echinacea* species in comparison with *Calendula officinalis*, without monitoring either the stimulated subpopulations of mononuclear cells or the separate activities of the plant extracts' individual compounds. Still, no statistically significant differences were observed between the plant species within the tested animals species, the SI induced by the two plant species being very similar for equine, bovine and goats. The variable in this respect was rather represented by the animals species than the plant taxonomy and its chemical composition.

The results obtained for the *C. officinalis* alcoholic extract, although statistically non significantly different from those induced by the *Echinacea* extracts within the frame of each animal species, were different between

horses, bovine and goats (Fig. 1, 2 and 3). Thus, the *Calendula* extract acted enhancing in horses and goats when compared to bovine.

4. Conclusion

When compared to the control, all extracts showed inhibiting effects in bovine and goats, but not in horses, at the tested doses, supporting host-based differences. The biological activity of the tested extracts was animal and plant species dependent, *Calendula officinalis* proving to better stimulate immune cell mitogenesis than *Echinacea* extracts, supporting differentiated effects of plants from different genera of the same family *Asteraceae*.

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Author Contribution

All authors declare equal contribution to the study design and experimental work, interpretation of the resultsand editing the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest during the accomplishment of this research. None of the authors has any financial and/or personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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ENVIRONMENTAL POLLUTION DEGREE CHANGES THE BIOLOGICAL ACTIVITY OF NETTLE PLANT EXTRACTS IN CHICKENS

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Abstract

The positive effects of plants, including stinging nettle (*Urtica dioica L.*) were reported by numerous researchers (1) as well as changes in the chemical composition of the plant due to heavy metal pollution (2, 3). This study aimed to compare the changes in body wight, total leukocyte numbers (TL) and delayed type hypersensitivity (DTH) in 28 days old chickens (n=12/group) injected subcutaneously twice (days 0 and 7), with 0.5 ml of alcoholic nettle extracts harvested from both unpolluted (group III) and polluted areas (group IV), against untreated (group I) and 70^0 alcohol treated (group II) controls. Blood was sampled three times, on days 0, 7 and 14, while the wattle test to a homologous lymphocyte suspension was performed on day 14 of the experiment.

There were no significant differences in weight gain/period between groups III (0.979 kg) and IV (0.967kg), against each other and controls (0.991 kg-group II and 1.029 kg - group I, respectively). As opposed to the slight changes of the TL in control groups, there was a constant increase in group III (from 15,400±3,421/mm^3 to 17,125±4,231/mm^3) and a decrease in group IV (17,611.1±2,401/mm^3 to 17,166.7±2,522/mm^3).

The wattle test results after 48 h were similar in groups I, II and IV, the differences ranging between 0.23 and 0.28 mm, while the unpolluted nettle extract diminished the DTH to a difference of 0.07 mm.

The polluted nettle extract induced the lowest weight gain and TL numbers, and increased the wattle reactivity when compared to the unpolluted one.

Keywords: *Urtica dioica L.,* chickens, weight, leukocytes, adaptive immunity, wattle test

1. Introduction

Urtica dioica (stinging nettle) is a plant well known for the medicinal effects of all its parts: roots, leaves and *herba*. Researches indicated that *U. dioica* L. could be considered as a natural alternative source for various fields of human activity (Yildirim et al., 2013). Characterization of the biological properties (antioxidant and antiradical) of phenolic compounds in nettle extracts is important for their possible applications in industrial areas, such as food/feed, cosmetics and also phytomedicine (Pinelli et al., 2008; Borsuk et al., 2011). Some studies propose that the therapeutic benefit of *U. dioica* extract could be partly attributed to its potential inhibition

of oxidative processes (Husein et al., 2014). Numerous therapeutic trials indicated that the use of various extracts from the plant provided beneficial effects in benign prostatic hyperplasia (Chrubasik, 2007), urinary tract problems, arthritis, neuralgia, cardiovascular disease, mild menorrhagia, gout, allergic rhinitis and others (Samakar et al., 2022).

In vitro assays indicate that *U. dioica* extract represented a significant source of natural alternatives to antimicrobial therapy, thus avoiding excessive antibiotic use (Simule, 2011). The potential employment as antimicrobial agent of this extract in pharmaceutical and food industry was also supported (Modarresi-Chahardehi et al., 2012). Thus, *Urtica dioica* leaves gave the best inhibitory activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Stanciuc et al., 2011). Crude extracts obtained by Soxhtlet extractor with ethyl acetate and hexane exhibited stronger antimicrobial effects against the Gram-positive bacteria than the Gram-negative bacteria (Modarresi-Chahardehi et al., 2012).

Immunological effects of *U. dioica* extracts have also been well documented. Dietary incorporation of *U. dioica* at 5% in *L. victorianus* (the ningu, a ray-finned fish in the family *Cyprinidae*) led to significantly higher relative percentage (95%) survival against *Aeromonas hydrophila* (Ngugi et al., 2015). Similarly, *Urtica dioica* agglutinin (UDA), which is a lectin found in stinging nettle, was capable of activating T-lymphocyte in a way not observed with any other known plant lectin (Galelli and Truffa-Bachi, 1993; Musette et al., 1996; Krystofova et al., 2010).

UDA appeared to prevent formation of a systemic lupus erythematosus-like condition in mice and has *in vitro* antiviral activity (Balzarini et al., 1992; Musette et al., 1996). UDA also antagonizes the epidermal growth factor receptor, a beneficial effect which could interfere with the pathogenesis of BPH (Wagnet et al., 1995). The stimulatory effect of *U. dioica* alcoholic extract on the phagocytic function in young, equine infectious anemia virus recently infected horses, was followed by an inhibitory effect which could be explained by the possible rapid carbon particle ingestion and an increase in cell membrane fragility (Bolfã et al., 2011; Borsuk et al., 2011).

The changes in the stinging nettle chemical composition due to heavy metal pollution were reported by numerous researchers (Notten et al., 2005; Krystofova et al., 2010). Based on studies regarding the extraction efficiency of metals in aqueous preparations, the elements in herb infusions were classified into highly-extractable (>55%)(K), moderately-extractable (20-55%)(Mg, Na, P, B, Zn and Cu) and poorly-extractable (<20%)(Al, Fe, Mn, Ba, Ca and Sr) (Pytlakowska et al., 2012).

Models were developed to estimate de heavy metal concentration in plants, *Urtica dioica* included, based on soil quality. The most important soil property that influenced *U. dioica* metal concentrations was the clay content, while pH affected Cu and Zn concentrations (Notten et al., 2005; Boshoff et al., 2014). Comparing in terms of heavy metal accumulation *T. officinale, U. dioica*, *R. pseudoacacia* and *M. recutita*, it was concluded that the first were better metal accumulators, while *M. recutita* was a metal avoider (Gjorgieva et al., 2011).

Other studies indicated that medicinal plants often represented subjected to heavy metal contamination exceeding permissible levels for some species and thus, collecting medicinal plants from contaminated area should be discouraged/banned [22]. Heavy metal ions in contaminated soils may easily enter the human food chain through crop plants, depending on geochemical characteristics of the soil and the ability of plants to selectively

accumulate some of these elements [23]. Prior to daily consumption of significant amounts of edible/medicinal plants, better compositional analyses should be performed (Mithril and Dragsted, 2012). This study aimed to investigate the biological activity of alcoholic extracts of nettle plants collected from diffrent areas known to be polluted (heavy autoroute traffic) and unpolluted (mountainy area) on chickens' growth rate and immunity.

2. Material and Methods

2.1. Plant material

Two alcoholic extracts of stinging nettle (*Urtica dioica* L.) were obtained by percolation, the plant material representing 9.5 g of the drug in each 100mL of tincture, by the Faculty of Pharmacy in Cluj-Napoca, according to the provisions of the General Pharmacopoeia. The stinging nettle plants were harvested from *a*) an unpolluted mountain region (SE Carpathian arch, Sibiu region) and *b*) from the edges of the city of Cluj-Napoca, considered to be an area polluted at a medium level, where exceedings of the maximum permissible concentration (MPC) of copper and lead, established by Order no. 756/1997 for the approval of the Requirements for environmental pollution assessment of the Romanian Government were recorded (Sarma et al., 2011).

2.2. Biological material

The experiment was carried out on 28 days old chickens, divided in four groups (n=12/group), injected subcutaneously twice (days 0 and 7), with 0.5 ml of alcoholic nettle extracts harvested from either unpolluted (group III) and polluted areas (group IV), against untreated, environmental and handling control (group I) and 70^0 alcohol treated, solvent control (group II). Blood was sampled three times, on days 0, 7 and 14, when the birds were weighed and leukocytes were counted (Türk method) (Davis et al., 2008), while the wattle test to a homologous (chicken) lymphocyte suspension was performed on day 14 of the experiment.

2.3. Lymphocyte suspension

Blood was sampled from conventionally farmed hens on heparin (50IU/ml, 5 ml)to prevent clotting, then centrifuged in density gradient on Ficoll® Paque Plus at 1500 rpm (Sigma Aldrich, 4 ml) and the buffy coat was separated. The cells were then washed in RPMI 1640, supplemented with 5% FCS and antibiotics (penicillin 1000IU/mL and streptomycine 1000μg/mL) by centrifugation at 1500 rpm (EBA 270, Hettich, Germany) for 10 min, three times and diluted to a final concentration of 4 x 10^6/ml in the supplemented RPMI 1640.

2.4. Delayed type hypersensitivity test

Wattle measurements using callipers were performed in each group, then the birds were injected with 0.1 ml of a chicken lymphocyte suspension in one of the wattles and RPMI 1640 in the other one.Both wattles were measured in each bird 48 h later.

Average values and standard deviation were calculated for all parameters, while the statistical significance of the differences between the polluted and unpolluted stinging nettle extracts were estimated by Student-t test.

3. Results and Discussion

Urtica dioica is a wide-spread herbaceous perrenial flowering plant of Order *Rosales*, Family *Urticaceae*, Genus *Urtica*. Numerous analyses of nettle plnt have revealed the presence of more than fifty different chemical constituents, showing anti-oxidative, anti-microbial, anti-ulcer and anesthetic capacity. The main immunologically active components were present in the roots of the plant, but the leaves also contained substantial amounts of poliphenols, known for their immunoactive effect (Samakar et al., 2022).

In the present study, no significant differences in weight gain/period between groups III (0.979 kg) and IV (0.967kg), against each other and controls (0.991 kg-group II and 1.029 kg - group I, respectively) were found (Fig 1).

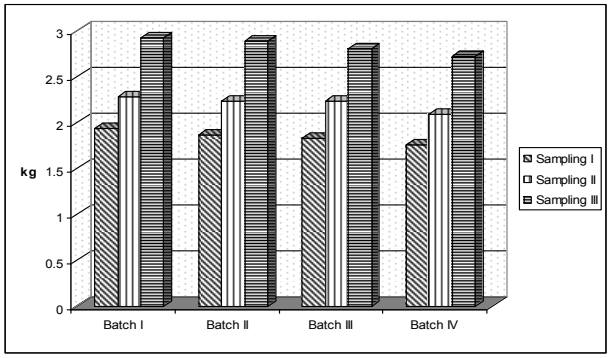

Figure 1: Dynamics of the body weight of chickens in the experimental groups (x ± s)(kg)

Although the birds in group IV had the least increase in weights, the treatment with the extract of stinging nettle collected from the polluted area did not significantly decrease the weight gain per the experimental period. As opposed to the slight changes of the TL in control groups (Fig 2), the parameter constantly increased in group III (from 15,400 ± 3,421/mm^3 to 17,125± 4,231/mm^3) and decreased in group IV (17,611.1 ± 2,401/mm3 to 17,166.7 ± 2,522/mm3) at 7 days.

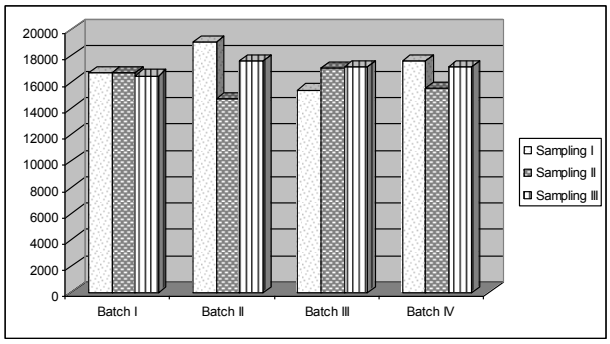

Figure 2: Total leukocyte numbers' development during the experimental period (x ± s)

The only statistically significant ($p < 0.05$) decrease was observed in the TL of group II, on day 7. Thus, the medium pollution level with metals on the edges of Cluj did not statistically significantly influence the body weight or the numbers of leukocytes.

Subsequently, the consumption of nettle plant from either unpolluted or polluted areas would not change the weights of chickens raised in either of the locations and would presumably preserve, with minor changes the immune response to microbial aggressors.

The wattle test results (Fig 3) after 48 h were similar in groups I, II and IV, the differences ranging between 0.23 and 0.28 mm, while the unpolluted nettle extract diminished the DTH to a difference of 0.07 mm.

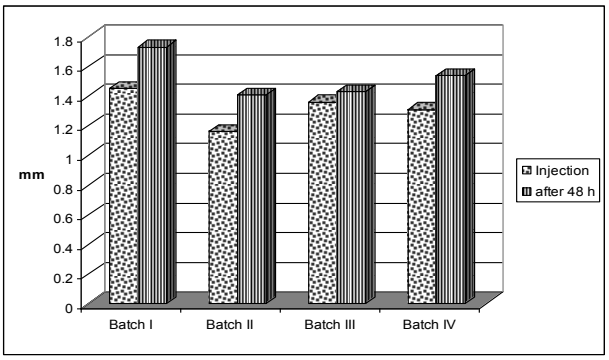

Figure 3: Wattle test responses subsequent to *in vivo* unpolluted and polluted nettle plant extract treatments in chickens (x ± s) (mm)

Pollution influenced to some extent the quality of the alcoholic nettle extracts and therefore their biological effects, , the functionality of leukocytes being changed by the pollution degree. Thus, the leukocytes from chickens treated with the nettle extract collected from the polluted area were more prone to respond to the agrression, exceding a normal response, while the non-polluted nettle extract preseved the DTH within a physiological range. Therefore such evaluations could be usefull for detecting an excessive aggression level and the response to it when the chickens are exposed to different levels of metal pollution. Further studies could elaborate a correlation between the heavy metal type and concentration and DTH, providing an evaluation grid.

4. Conclusion

In spite of the fact that, based on literature, *U. dioica* seems to be a heavy metal accumulator, the results of this experiment did not indicate an influence of the officially reported medium pollution level in the soil of Cluj-Napoca surroundings or the lack of pollution in the mountains, at the site of the nettle plant collection, sufficient to induce statistically significant changes in the food conversion rate, numbers of leukocytes or their adaptive function in the graft rejection test. Further studies could explicit, if present, a dose-effect correlation.

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Author Contribution

All authors declare equal contribution to the study design and experimental work, interpretation of the resultsand editing the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest during the accomplishment of this research. None of the authors has any financial and/or personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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Herbal Tea Remedies : Exploring the Ethnobotanical Landscape of Medicinal Plants in Infants and Child Healthcare

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Abstract

Herbal medicine occupies a prominent position as one of the most ancient and globally recognized forms of medical treatment. It offers a compelling alternative for achieving healing and recovery without the potential risk of generating new ailments. Despite the advancements made in conventional medicine, the increasing number of medical practitioners, and the establishment of social security systems, many parents have turned to herbal medicine when searching for remedies for their children. The demand for "natural remedies" perceived as being "risk-free" has been steadily rising. Among the accessible methods of harnessing the benefits of herbal medicine, herbal teas have garnered significant popularity. Consequently, our study aims to acquire comprehensive information pertaining to the composition, methods of administration, and diverse plant varieties employed in these herbal teas. Additionally, we seek to ascertain their positive effects and potential toxicity. The current research encompasses an ethnobotanical survey conducted over a period of one month, spanning from January 6, 2021, to February 19, 2021. The survey involved 753 respondents, predominantly consisting of primiparous or multiparous mothers, who actively participated by completing an electronic questionnaire. The findings reveal that a total of 684 mothers utilized herbal teas for their infants, employing 55 herbal remedies to address various indications. The most frequently sought-after effects included carminative properties and relief from colic. Moreover, 9% of the users reported experiencing adverse effects associated with the administration of herbal teas to their infants. In conclusion, the utilization of herbal teas as a natural remedy by Algerian mothers is widely embraced; however, it is crucial to recognize that the "natural" attribute of plants does not automatically guarantee their harmless nature.

Key Words: Herbal medicine, Herbal teas, Children, Mothers, Medicinal plants

1. Introduction

Medicinal plants have retained the prestige of the oldest remedies and continue to possess an inexhaustible potential. The plant world offers abundant and diverse raw materials to therapeutics, which are utilized in phytotherapy.

Phytotherapy is one of the oldest forms of medicine in the world. It represents an interesting alternative for treatment and healing without creating new diseases. Despite the phenomenal development of the pharmaceutical and chemical industry, the popular interest in phytotherapy has never

ceased to evolve. Nowadays, these two forms of medication are closely intertwined since the molecular models of most market-available drugs originate from plants (Belkacem S., 2009).

In developing countries, between 70 and 95% of the population resorts to medicinal plants for primary healthcare due to a lack of access to prescribed medications and because plants have demonstrated genuine efficacy. It is estimated that at least 25% of all modern medicines are directly or indirectly derive from plants thanks to the application of modern technologies to traditional knowledge. (Nace International., 2007) Furthermore, the side effects induced by medications concern users, leading them to seek less aggressive treatments.

Despite the progress of medicine, the increase in the number of doctors, and the establishment of social security, many parents turn to phytotherapy for their children. They increasingly seek "natural remedies" believed to be "risk-free." However, the "natural" nature of plants does not guarantee their harmlessness (Rombaux-gilleron P., 2017).

Herbal tea is a highly accessible means of enjoying the benefits of phytotherapy. It involves extracting aromatic compounds from plants through various preparation methods such as maceration, decoction, or infusion of plant material (fresh or dried flowers, stems, roots, leaves), usually in hot water.

However, the administration of herbal teas to an infant can have serious consequences on their health.

2. Material and Methods

2.1. Objectives of the study

The main objective was to investigate whether women frequently give herbal teas to their children during their first months of life and obtain detailed information about the composition and mode of administration of these herbal teas.

As secondary objective :

- Stady the sociodemographic and epidemiological profile of mothers
- Identufy mothers orientation modalities
- Identify the various adverse effects observed in children following the administration of herbal teas

2.2. Type of study

This was a descriptive retrospective study conducted over a one-month period (from January 6, 2021, to February 19, 2021), involving both primiparous and multiparous mothers.

2.3. Study population

2.3.1. Inclusion criteria

- Algerian mothers.
- Aged over 18 years.
- Primiparous and multiparous.
- Using herbal teas for their children.

2.3.2. Non-inclusion criteria

- Mothers not using herbal teas for their children.

2.4. Recruitment of mothers

The recruitment of mothers took place at the offices of specialist pediatric doctors who responded to our questionnaire, which was presented in paper format and through an electronic questionnaire created using Google Forms® on the social media platform Facebook. The questionnaire consisted of three sections:

- Mother's profile
- Data on the consumption of herbal teas in children
- Data on the mothers' opinions on herbal teas

2.5. Ethical aspect

The duration of the interview ranged from 10 to 15 minutes. The questionnaire was anonymous, discreet, and unbiased. It was filled out by the mothers alone to ensure their responses were as honest as possible.

2.6. Statistical analysis of data

The data was entered into the MS Excel® software. Quantitative variables were expressed in frequencies, while qualitative variables were presented as percentages.

3. Results and Discussion

3.1. Descriptive parameters of the population (mothers)

3.1.1. Age groups

The age of the mothers ranged from 16 to 65 years, with the highest response rate in the 26-30 age group (36.38%), followed by the 31-35 age group (28.02%) (Figure 1).

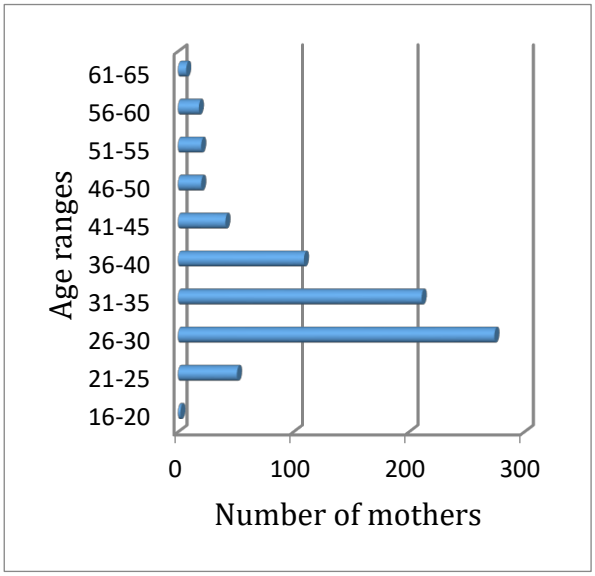

Figure 1. Distribution of the population by age groups.

This result aligns with Valérie Huet's study in Reunion Island in 2010, where 79% of the mothers were between 26-35 years old (Huet V., 2010).

3.1.2. The number of childrens

This ethnobotanical survey involved 753 mothers.
323 mothers (42.9%) are uniparous, followed by 238 mothers (31.6%) are biparous; and 116 mothers (15.4%) have 3 children and the remaining 10.09% have more than 3 children (Figure 2).

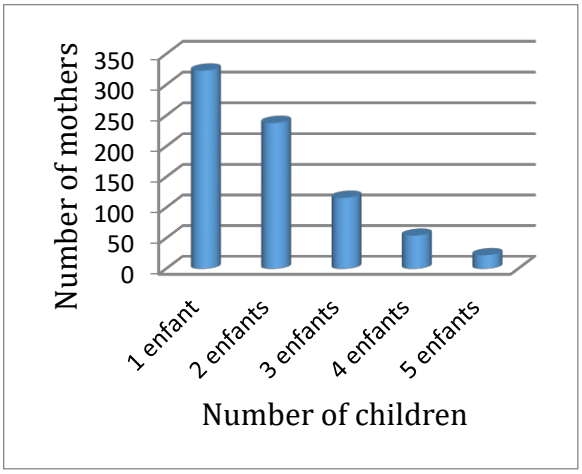

Figure 2. Distribution of the population by number of children.

3.1.3. Educational level

In terms of education level, among all the herbal tea users, the majority were university-educated, accounting for 80% (Figure 3).

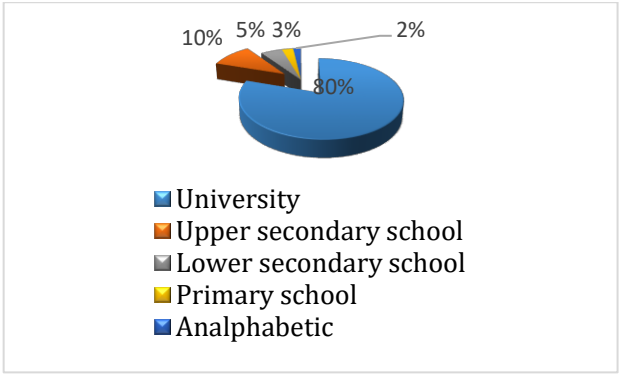

Figure 3. Distibution of the population by educational level.

This result is similar to the findings of other researchers who conducted studies in the United States (Ottolini MC et al., 2001),

Canada (Spigelblatt Let al., 1994), and Italy (Menniti-Ippolito F et al ., 2002).

Comparatively, this result is supported by two studies conducted in Turkey (Ozturk et al., 2008) and Germany (Du Y et al., 2014), which reported that the use of phytotherapy in children is associated with a higher levelof education of the mother.

All these previous studies demonstrate that medicinal plants can be dangerous when used unknowingly, particularly among individuals with low levels of education who use medicinal plants irrationally. Illiterate individuals may not fully understand the verbal instructions given by herbalists and healers. However, the rate of illiteracy is significantly lower in our population among users of medicinal plants.

3.1.4. Habitat area

The majority of mothers belong to urban areas (91%) (Figure 4).

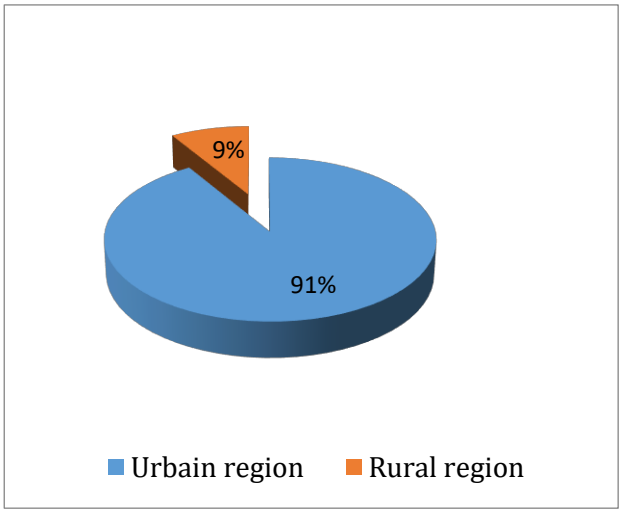

Figure 4. Distribution of population by habitat area.

This distribution can be explained by the predominantly urban population that responded to our questionnaire.

3.2. Descriptive parameters of herbal tea consumption among children

3.2.1. Herbal tea usage by mothers

This ethnobotanical survey involved 753 mothers, 91% of whom used herbal teas for their children (Figure 5).

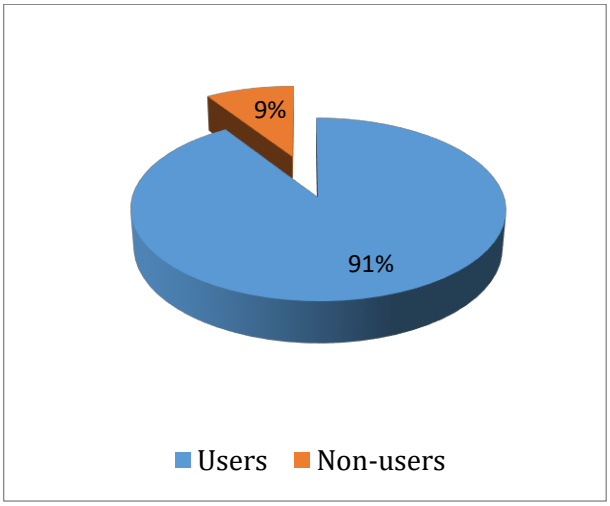

Figure 5. Distribution of the population by herbal tea usage.

This result is comparable to Mkedder study conducted in Tlemcen in 2018 on the use of phytotherapy in children, where the usage rate was 68% (Mkedder et al ., 2018). Similarly, a study conducted in Reunion Island by Huet in 2010 on the administration of herbal teas to breastfed babies in the Creole society revealed that 79% of the interviewed mothers had given at least one herbal tea to their previous children (Huet V.,2010). High rates of phytotherapy use in children have also been reported in studies conducted in Mali (96.7%)(Chaka O., 2014), Germany (85.5%)(Hümer et al., 2010), and Turkey (76.7%)(Ozturk et al., 2008). However, this rate is much lower in the United States (10%)(Loman, D.G., 2003). This study confirms the widespread use of traditional remedies in children despite the availability and accessibility of modern medicine.

3.2.2. Types of herbal teas used

Almost two-thirds of our population (58%) use loose herbs in the preparation of herbal teas for their children and 20% of the population use packaged herbal teas and 22% use both (Figure 6).

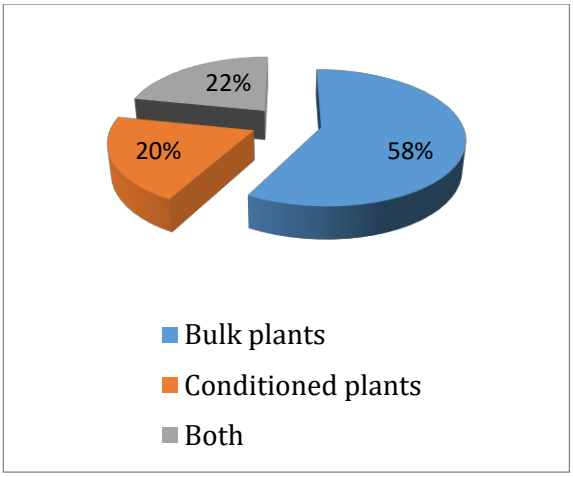

Figure 6. Distribution of polulation by the types of herbal teas used.

3.2.3. Number of plants used in the herbal teas

The use of medicinal plants for different treatments is not always singular, but mothers often resort to a mixture of several species for a given treatment. Moreover, a single species can be used to treat more than one symptom.

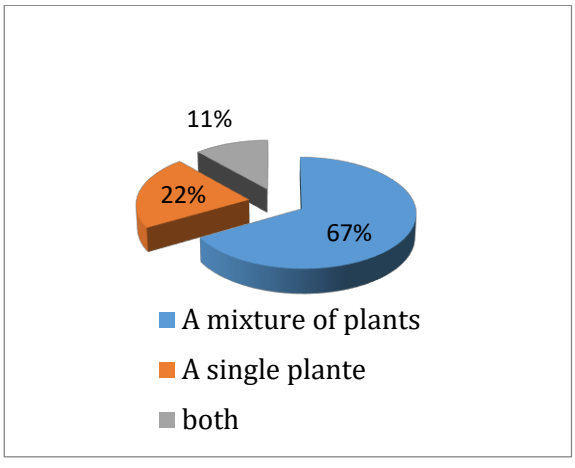

Figure 7. Distribution of the population by number of plants used.

In our study, more than half of the population (68%) use a single plant in their herbal teas, while 22% use herbal teas composed of a mixture of plants, and 11% of the population use herbal teas made from a single plant and herbal teas based on a mixture of plants (Figure 7).

This result aligns with Mkedder's study conducted in Tlemcen, which shows that for the majority of preparations (83.8%), plants are used alone(Mkedder et al., 2018). Similar findings were observed in studies conducted in Germany (Du et al., 2014) and Ghana (Asase, A., 2014).

The World Journal of Gastroenterology (WJG) in 2012 has already addressed this type of usage and concluded that herbal treatment for functional gastrointestinal disorders is generally not a single preparation, as an extract from a single herb is claimed to be ineffective. Therefore, many preparations are provided in the form of a combination of drugs. According to traditional Chinese philosophy of maintaining and restoring balance, certain herbs are suggested to treat the main disease, while others are used to enhance the therapeutic effect and reduce the toxicity of the main drugs, and some have a harmonizing effect to guide the drugs to the appropriate organs (Rahimi et al., 2012).

3.2.4. The plants used

A total of 55 medicinal plants were identified, distributed among 23 families (Table X).

The most represented families are: Lamiaceae (12 plants), Apiaceae (8 plants), Asteraceae, Rosaceae, Myrtaceae, Rutaceae, and Poaceae (three plants each), Malvaceae, Fabaceae, Lauraceae, and Zingiberaceae (two plants each), Verbena-ceae, Rhamnaceae, Schisandraceae, Caprifoliaceae, Linaceae, Iridaceae, Brassicaceae, Cupressaceae, Punicaceae, and Theaceae (one plant each).

This result is similar to Mkedder's study in the Tlemcen region of Algeria, which identified 51 plant species distributed among 26 botanical families, with the most frequent families being Lamiaceae, Apiaceae, and Fabaceae, mainly used for digestive and respiratory disorders(Mkedder et al., 2018). Comparing these results with various ethnobotanical studies on the use of medicinal plants, we found quite similar results: Lamiaceae and Apiaceae often appear in studies conducted in Tlemcen (Elyebedri et al., 2017), Tiaret (Miara et al .,2013), and Morocco (Hseini et al.,2007) .

Out of all the obtained results, we have compiled the most commonly used medicinal plants by the local population. Most of these plants grow spontaneously in the studied region, including *Foeniculum vulgare* Miller, *Cuminum cyminum* L, *Pimpinella anisum* L, *Lippia citriodora* H, B, K, *Mentha viridis* L, *Trigonella foenum graecum* L, *Carum carvi* L, *Anthemis nobilis* L, *Citrus aurantium* L, and *Tilia platyphyllos* Scop.

The most frequently mentioned plant is *Foeniculum vulgare* Miller (65.2%), followed by *Cuminum cyminum* L (56.58%), *Pimpinella anisum* L (47.66%), *Lippia citriodora* H, B, K (28.36%), *Mentha viridis* L (14.18%), *Trigonella foenum graecum* L (12.72%), *Carum carvi* L (11.11%), *Anthemis nobilis* L (9.36%), *Citrus aurantium* L (6.29%), and *Tilia platyphyllos* Scop (4.39%).

The mentioned medicinal plants are distributed among 23 families. The most represented families are Lamiaceae (12 plants) and Apiaceae (8 plants). The used parts of Lamiaceae are represented by leaves, flowers, and flowering tops, while the dried fruits are used for Apiaceae. This difference in the used parts of the plants is justified by the variability in the concentration of active principles in each plant organ or even each species.

The dominance of leaves is justified by the fact that they are the site of the majority of photochemical reactions and reservoirs of derived organic matter (Babba Aissa F., 1999).. Leaves provide the majority of alkaloids, glycosides, and essential oils. The importance of fruits is due to the concentration of their bitter, carbohydrate, or aromatic substances associated with certain pigments that give them a characteristic coloration. The use of flowers is due to their richness in essential oils. The same applies to roots and seeds, which are rich in sugars and vitamins (Asase et al., 2014).

The dominance of leaves was confirmed by Mkedder's study, where the majority of preparations mentioned were decoctions or infusions of plants, especially leaves (Mkedder et al., 2018), as well as other studies conducted in children in Ghana (Asase et al., 2014) and Burkina Faso (Zerbo et al., 2008).

3.2.5. Indications cited

The ethnobotanical analysis allowed for the identification of several diseases treated with medicinal plants. In general, the obtained results show that the most sought-after indications for treatment are gas and bloating with a rate of 47.51%, followed by colic (34.8%) (Table 1). This result is consistent with the results of surveys conducted in Tiaret (Miara et al., 2013), Tlemcen (Elyebedri et al., 2017) and Morocco (Hseini et al.,2007) and also the study by (Huet V.,2010), where herbal teas were frequently indicated to relieve colic in infants, In contrast to Turkish and American parents where half of the parents reported using herbal medicines to relieve respiratory problems in their children (Ozturk et al., 2008) (Pitetti et al., 2001).

Table 1. Indication cited and their repetitions frequencies

Indications	Occurrences	Repetition frequencies%
Gas	325	47,51
Colic	238	34,8
Carminative	129	18,85
Sedative	104	15,2
Abdominal pains	89	13,01
Flu	47	6,87
Constipation	46	6,73
Diarrhea	24	3,51
Cold	17	2,49
Baloating	16	2,34
Vomiting	16	2,33
Fever	15	2,19
Appetite stimulant	15	2,19
Toothache	11	1,6
Facilitate digestion	10	1,46
Coughs	11	1,61
Respiratory illness	4	0,58
Weight gain	4	0,58
Anti-spasmodic	3	0,43
Soothe the baby	2	0,29
Sweet taste appreciated by baby	2	0,29
Allergy	2	0,29
Well being	2	0,29
Gastric problems	2	0,43
Hydration	2	0,29
Anthelmintic	1	0,15
Nausea	2	0,30
Jaundice	1	0,15
Nasopharyngitis	1	0,15
Digestive problens	1	0,15
Infection	1	0,15
Tonsillitis	1	0,15
Nasal discharge	1	0,15
Flatulence	1	0,15
Immunized	1	0,15
Suckling	1	0,15
Sputum	1	0,15
Intoxication	1	0,15

The majority of the indications cited for plants frequently used in the region are consistent with the literature. This is valid for almost all the species cited for certain pathologies, in particular digestive pathologies, the indications most cited by this population of mothers were the subject of a bibliographical search which made it possible to identify the following points: According to Commission E (scientific advisory board of the German Food and Drug Administration) ¸ aniseed in monotherapy or in combination has the following indications: dyspeptic disorders and bloating and the following properties are recognised: spasmolytic and antibacterial (Babulka P., 2004). A clinical herbal medicine article indicated that the treatment of irritable bowel is based on a 15-minute infusion of anise seeds (*Pimpinella anisum*), fennel seeds (*Foeniculum vulgare*), and hawthorn fruits (*Cratægus monogyna*) at 10 g (Goetz P., 2014). For caraway, several studies have been done to evaluate their effects: one demonstrated the antioxidant activity of *Cuminum cyminum*, and antimicrobial activity against most of the strains tested (Athamena et al., 2009). The accepted medical indications for caraway: digestive disorders such as gastrointestinal dyspepsia, flatulence, eructation, epigastric bloating, moderate spasms of the intestinal tract, colon infection (adjunctive treatment), irritable bowel and spastic colopathy (Ghédira et al., 2016). For the treatment of insomnia *Carum carvi* and *Cuminum cyminum* are wrongly used, *Pimpinella anisum* which is mostly used has a hypnotic and central nervous system depressant effect at high doses.

3.2.6. Use of herbal teas according to the orientation modalities

In our study, 28.42% of mothers use herbal teas based on their own initiative, 26.56% use them based on their doctor's advice, 14.48% use them after consulting a

pharmacist, and finally, 3.98% of mothers use them following advice from an herbalist (Figure 8).

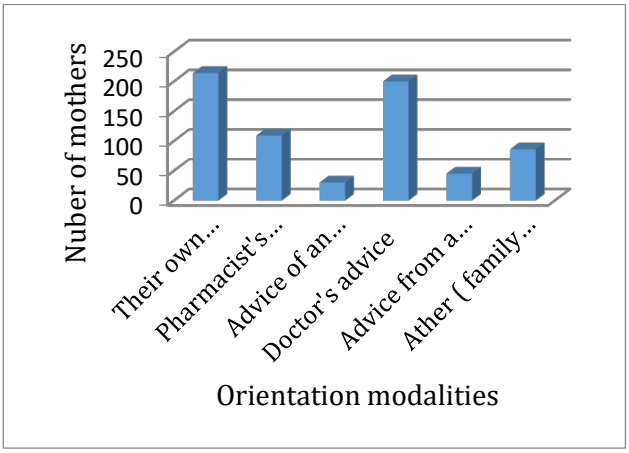

Figure 8. Distribution of population according to their orientation modalities.

In contrast, Mkedder's study conducted in Tlemcen showed that in 84.3% of cases, the origin of the information was based on the experience of others, and the use of herbalists remained minimal in the region (2.4%) (Mkedder et al., 2018). This origin is also predominant in studies conducted in Turkey (Ozturk, et al. (2008), Germany (Weissenstein, A., et al., (2013), England (Ernst, E., et al 2002), and Morocco(Bouayyadi, L., et al 2015). In contrast, Valérie HUET's study in Reunion Island showed that 89.47% of mothers did not seek advice from a healthcare professional (Huet V,. 2010). Therefore, knowledge of medicinal plant usage is generally acquired through accumulated experience passed down from one generation to another. The transmission of this knowledge is currently at risk because it is not always guaranteed (Anyinam C., 1995).

3.2.7. Places of purchase of herbal teas

Nearly half, 388 (43.29%) of our population, purchase their herbal teas or their constituents from an herbalist, followed by 256 (33.99%) who buy them from a pharmacy, and 40 (5.31%) who buy them from a natural products store (Figure 9).

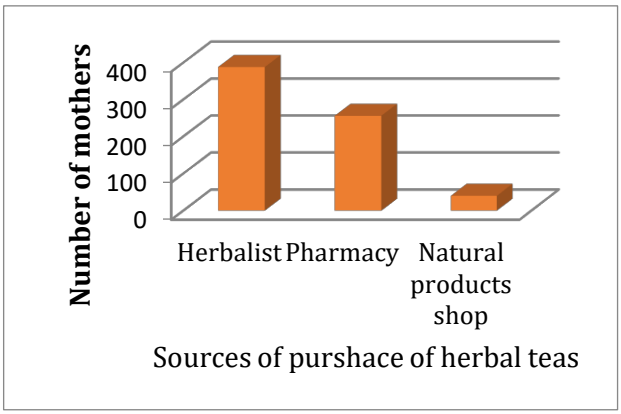

Figure 9. Distribution of the population according to different sources of herbal tea purchase.

3.2.8. Methods of preparation of herbal Teas

The most applied method for preparing herbal teas by our population is infusion (63.87%), followed by decoction (24.03%), with maceration accounting for 2.92% (Figure 10).

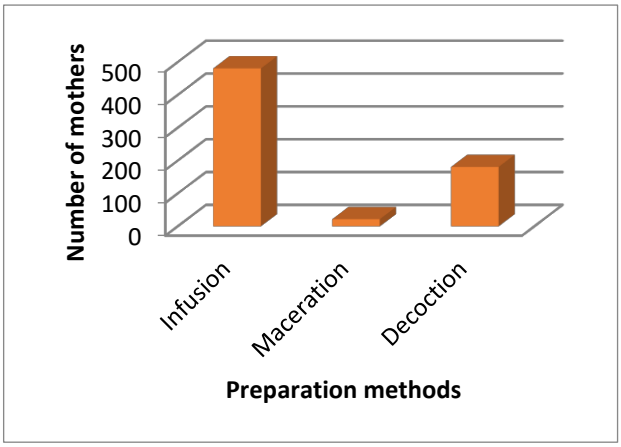

Figure 10. Distribution of the population according to their methods of preparing herbal teas.

According to (Salhi et al, 2010) , users always seek the simplest method to prepare herbal medicines, confirming the dominance of the "infusion" method in our case (Diatta et al., 2013).
The best use of a plant is that which preserves all its properties while allowing the

extraction and assimilation of the active principles (Dextreit , R., 1984) .In addition to being the preparation method that preserves the active principles of the plant (El Hafian et al., 2014), the decoction warms the body and disinfects the plant to neutralize the toxic effect of certain recipes, but it can destroy certain active principles of the species used.

All the prepared recipes are administered orally (herbal teas) because it represents the simplest, most effective, and quickest route of administration. Similar results were observed in a study by El Hafian et al. (2014), which found that oral administration, including various preparation methods (infusion, maceration, decoction, herbal tea, internal powder), is the most recommended method, as well as another study in Ghana (Asase et al., 2014).

3.2.9. Frequency of use of herbal teas

76% of the mothers use herbal teas regularly for their children (Figure 11).

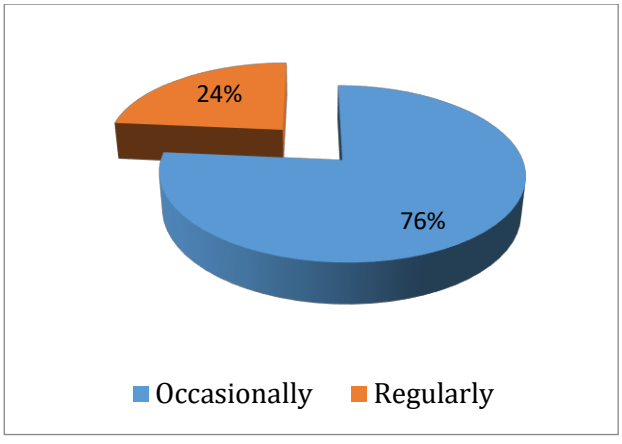

Figure 11. Distribution of the population according to their frequency of herbal tea use.

3.2.10. Ages of children at the time of the first administration of herbal tea

In our survey, the majority of mothers have given herbal teas to their infants since birth, which is consistent with Huet's study (Huet V., 2010) (Table 2).

Table 2. Ages of children at the time of the first administration of herbal tea.

Age of the infant at the time of the first intake	Occurrences	Repetition frequencies %
From birth	58	8,48
Threedays	1	0,15
Ten days	6	0,88
A few days	2	0,29
After 40 days	1	0,15
One week	40	5,85
Two weeks	11	1,61
Three weeks	5	0,73
One month	170	24,85
Two months	50	7,3
Three months	61	8,91
Four months	11	1,61
Five months	4	0,58
Six months	39	5,7
Ten months	2	0,29
Eighteen months	2	0,29
One year	15	2,19
Two years	4	0,58
Three years	3	0,44
Four years	1	0,15
Five years	2	0,29
Did not respond	196	28,65

3.2.11. Duration of use

The duration of treatment varied, with the most frequent duration being indefinite (no age limitation) at 18.86%, followed by 6.87% for a duration of three months, 6.58% for one month, 4.97% for 12 months, 3.5% for two months, 2.77% for a few months, and finally 2.04% for a few days (Table 3).

Table 3. Duration of use of herbal teas for children .

Duration of use	Occurrences	Repetition frequencies %
Two days	10	1,46
Four days	14	2,04
Ten days	4	0,58
Twenty days	1	0,15
A few days	18	2,63
One week	8	1,16
Two weeks	3	0,44
A few weeks	1	0,15
One month	45	6,58
Two months	24	3,5
Three months	47	6,87
Four months	19	2,78
Five months	7	1,02
Six months	31	4,53
Seven months	3	0,43
Ten months	7	1,02
Twelve months	34	4,97
Eighteen months	6	0,87
A few months	14	2,04
The first months	19	2,77
two years	14	2,05
Three years	6	0,88
Five years	1	0,15
Six years	2	0,29
Seven years	2	0,29
Nine years	1	0,15
Indefinite duration (no age limit)	129	18,86
Did not respond	214	0,31

This result is consistent with Mkedder's study, where long-term use accounted for 13.70% and 16.40% until recovery (Mkedder et al., 2018). It is also in line with studies conducted in Germany (Du et al., 2014) and Huet's study, where the majority of mothers administered herbal teas for a short period of time (Huet V., 2010).

4. Descriptive parameters of mothers' opinions on herbal teas

4.1. Observation of adverse effects in children following the administration of herbal teas

91% of the population did not notice any adverse effects following the use of herbal teas for their infants and children (Figure 12).

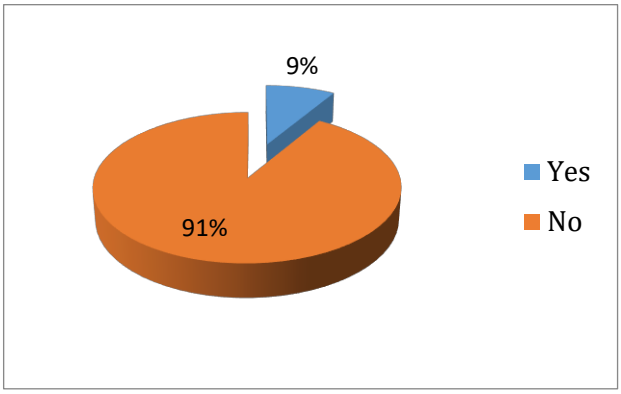

Figure 12. Distribution of the population according to their observations of adverse effects.

This is in line with the Tlemcen study, where few mothers reported the appearance of adverse effects following the use of herbs for their children (Mkedder et al., 2018).

4.2. Cited adverse effects

For the 9% who noticed adverse effects following the use of herbal teas, the frequency of recurrence of the adverse effect diarrhea was the highest at 25.42%, followed by 8.47% for weight loss and satiety, 6.78% for deep sleep, vomiting, disruption of breastfeeding and stool discoloration and many other lesser cited adverse effects (Table 4).

This result was demonstrated by Benkhnigue's 2011 study which found that 8% of people believe that herbal treatment causes side effects, toxicity and even worsening of disease (Benkhnigue O., 2011).

Table 4. Adverse effects noticed by mothers in their children

Cited side effects	Occurrences	Repetition frequencies %
Diarrhoea	15	25,42
Weight loss	5	8,47
Satiation	5	8,47
Green stool coloration	4	6,78
Vomiting	4	6,78
Disruption of breastfeeding due to reduced feeding frequency	4	6,78
Long sleep	4	6,78
Lack of gas	3	5,08
Colic	2	3,39
Constipation	2	3,39
Digestive discomfort	1	1,69
Kidney problems	1	1,69
Anemia	1	1,69
Feeling of hunger	1	1,69
Drowsiness	1	1,69
Expectorant	1	1,69
Regurgitation	1	1,69
Agitation	1	1,69
Abdominal pain	1	1,69
Shortness of breath	1	1,69
Digestive system disorder	1	1,69

Studies on the adverse effects of herbal medicine show that most adverse effects of medicinal plants are reported not from the plant itself, but from misidentification, unintentional contamination (by another plant, by heavy metals, by pathogenic micro-organisms or by agrochemical residues), failure to take the correct dose or interaction with drugs. Thus all plants should be considered a priori dangerous, even those that seem particularly well tamed by humans should still be viewed with suspicion (Delaveau P., 1977).

4.3. Reports of health problems related to the administration of herbal teas by mothers

87% of our population have heard of health problems related to the administration of herbal tea to infants and children (Figure 13).

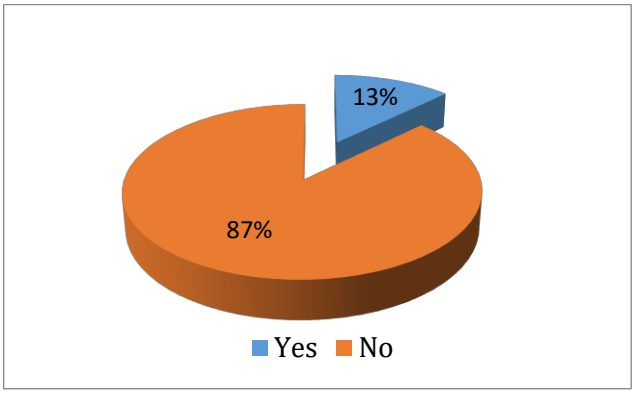

Figure 13. Distribution of the population according to their reports of health problems related to the administration of herbal teas.

The same applies to the feedback from mothers on health problems related to the use of herbal teas

4.4. Health problems reported

For those who have heard of health problems related to the administration of herbal teas, the frequency of recurrence of kidney problems was the highest at 29.88% (Table 5). This result was demonstrated by Chebat in 2014 on Risk assessment of adverse events related to the use of herbal medicines in children with haematological and cancerous diseases showed that among the patients surveyed, using herbal medicines, it was noted that 9.4% had experienced adverse events. Analysis of the results showed that tubulointerstitial nephritis was the most frequent adverse effect (3.2%), followed by cough and cold (0.5%), diarrhoea (0.2%) and vomiting (0.2%) (Chebat et al., 2014).

Table 5 . Reported health problems

Health problems	Occurrences	Repetition frequencies %
Kidney problems	26	29,88
Diarrhea	12	13,79
Intestinal problems	9	10,34
Digestive problems	9	10,34
Gastric problems	8	9,2
Vomiting	6	6,9
Intoxication due to overdose	5	5,75
Satiation (refusal to breastfeed)	9	10,35
Allergy	4	4,6
Nervous system disorders	4	4,6
Early weaning from breastfeeding	4	4,6
Destruction of intestinal flora	3	3,45
Weight loss	3	3,45
Weakness	3	3,45
Insomnia	2	2,3
Anemia	2	2,3
Relaxation of stomach muscles	2	2,3
Intoxication due to toxic plants	2	2,3
Liver problems	2	2,3
Renal colic	2	2,3
Jaundice	1	1,15
Intelligence disorders	1	1,15
Abdominal cramps	1	1,15
Convulsion	1	1,15
Sleep disturbance	1	1,15
Malnourishment	1	1,15
Stomach stuffing	1	1,15
Anesthetic effect	1	1,15
Sedative effect	1	1,15
Intussusception	1	1,15
Colopathy	1	1,15
Bloody stools	1	1,15
Intestinal inflammation	1	1,15
Hemorrhage	1	1,15
Drowsiness	1	1,15
Swallowing problem	1	1,15
Body disorder	1	1,15
Vertigo	1	1,15

4.5. Trust in terms of quality attributed to herbal teas

82% of our population trust herbal teas in terms of quality (Figure 14).

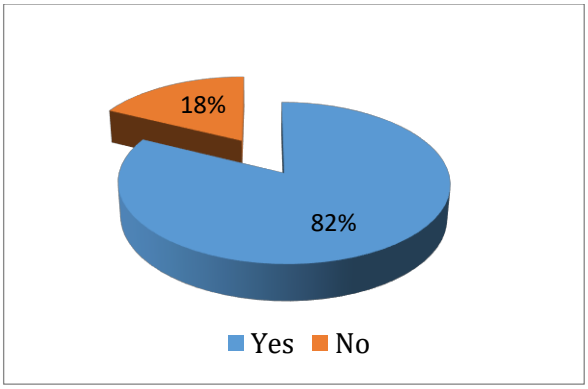

Figure 14. Distribution of the population according to their trust in herbal teas in terms of quality.

4.6. Type of herbal teas they trust more

More than half (64%) of our population trust non-packaged herbal teas (prepared from loose herbs) more (Figure 15).

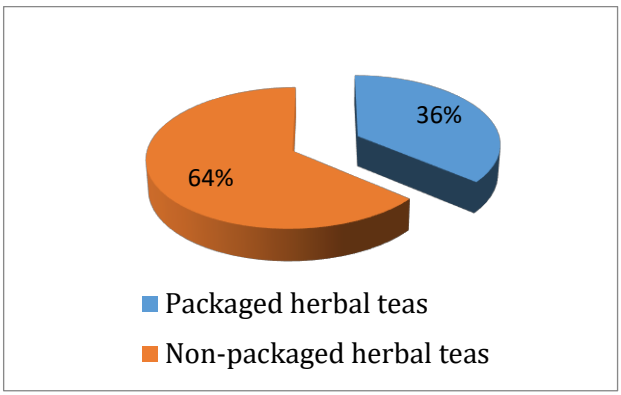

Figure 15. Distribution of the population according to the type of herbal teas they trust more.

4.7. Preferences for places to purchase herbal teas in terms of quality

More than half of our population, 395 (52.46%), trust herbal teas purchased at pharmacies, while 231 (30.68%) trust herbal

teas purchased from herbalists, and 58 (7.70%) trust herbal teas purchased from natural product stores (Figure 16).

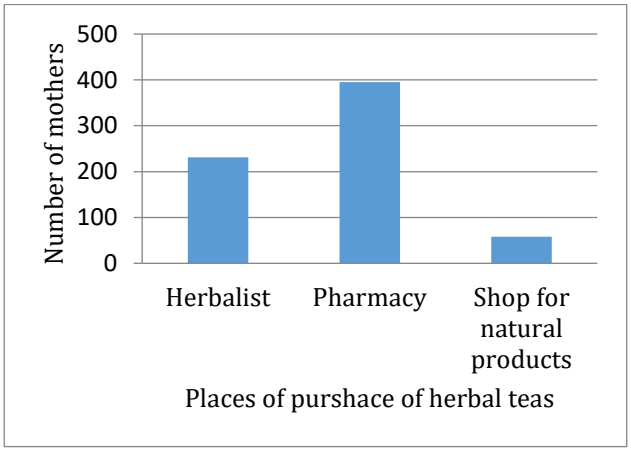

Figure 16. Distribution of the population according to their preferences for places to purchase herbal teas in terms of quality.

4.8. Preferred types of herbal teas in terms of price

It is observed that 74% of our population prefer non-packaged herbal teas prepared at home from loose herbs in terms of price (Figure 17).

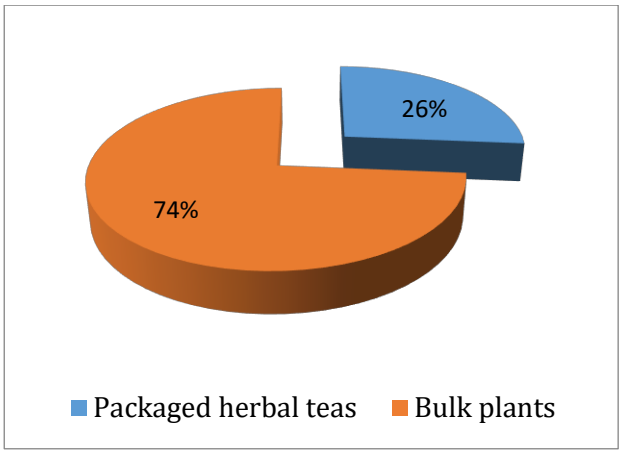

Figure 17. Distribution of the population according to their preferences in terms of price.

4.9. Preferences for places to purchase herbal teas in terms of price

386 of our population (58%) prefer herbal teas purchased from herbalists in terms of price (Figure 18).

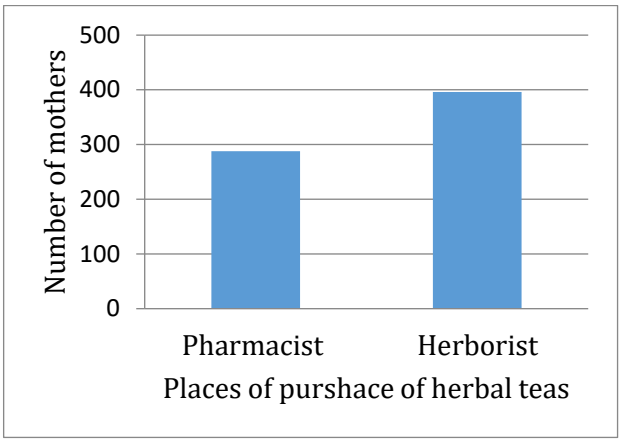

Figure 18. Distribution of the population according to their preferences for places to purchase herbal teas in terms of price.

This result coincides with results obtained by Hmamouchi in 2012 in Morocco, which showed that 62% of people saw that traditional medicine was more effective against pain. 21% of other respondents prefer traditional treatment because of its easy acquisition, 15.67% prefer it because it is economical. While 8.33% of people find medication to be ineffective and more harmful than helpful. (Hmamouchi I., 2012)

This can be explained by the fact that the choice of herbal medicine by the local population is most often related to the isolation of rural areas, non-existence or rudimentary state of health infrastructure, high cost of pharmaceutical products and low income (Guedje N.M., 2011)

Table X. The mentioned indications and their repetion frequencies

Plants	Scientific names	Arabic names	Families	Parts used	Counts	Repetition frequencies %
Green anise	*Pimpinella anisum* L	حبة الحلاوة	APIACEAE	Dried fruit	326	47,66
Fennel	*Foeniculum vulgare* Miller	بسباس , الشمر , النافع	APIACEAE	Dried fruit	446	65,2
Cumin	*Cuminum cyminum* L	كمون	APIACEAE	Dried fruit	387	56,58
Roman chamomile	*Anthemis nobilis* L	بابونج	ASTERACEAE	Flower head	64	9,36
Lemon Verbena	*Lippia citriodora* H,B et K	اللويزة , الطرنجية	VERBENACEAE	Leaves	194	28,36
Linden	*Tilia platyphyllos* Scop	زيزفون	MALVACEAE	Inflorescence and bract	30	4,39
Orange-tree	*Citrus aurantium* L	شجر البرتقال	RUTACEAE	Barks, Leaves, Flowers	43	6,29
Spearmint	*Mentha viridis* L	نعناع	LAMIACEAE	Leaves, Flowering tops	97	14,18
Medlar	*Eriobotrya japonica* (Thunb)Lindl	بوعضيمة , الزعرور	ROSACEAE	Leaves	2	0,29
Peach-tree	*Prunus persica* (L)Batsch	الخوخ	ROSACEAE	Leaves	1	0,15
Common thyme	*Thymus vulgaris* L	زعيترة	LAMIACEAE	Leaves, Flowering tops	48	7,02
White wormwood	*Artemisia herba alba* Asso	الشيح	ASTERACEAE	Aerial part	11	1,61
Fenugreek	*Trigonella foenum graecum* L	حلبة	FABACEAE	Seeds	87	12,72
Licorice root	*Glycyrrhiza glabra* L	عرق السوس	FABACEAE	Rhizomes, root	22	3,22
Caraway	*Carum carvi* L	الكروية	APIACEAE	Dried fruit	76	11,11
Curry Tree	*Murraya koenigii* (L) Spreng	الكاري	RUTACEAE	Roots, Barks, Leaves	1	0,15
Lemon balm	*Melissa officinalis* L	ترنجاني , نعناع الترنج	LAMIACEAE	Leaves, Stems, Flowers	4	0,58
Mushrooms				Dried mushrooms	1	0,15
Celery	*Apium graveolens* L	كرفس	APIACEAE	stems, seeds	6	0,88
Alaternus	*Rhamnus alaternus* L	مليلس	RHAMNACEAE	Leaves	2	0,29
Hawthorn	*Crataegus oxyacantha* L	بابا عجينة, بوخرورو	ROSACEAE	Flowering tops	1	0,15

Chinese star anise	*Illicium verum* Hook. Fil	نجم الأرض, جاذبة , باديان	SCHISANDRACEAE	Dried fruit	4	0,58
Common laurel	*Laurus nobilis* L	الرند	LAURACEAE	Leavers	9	1,32
Valerian	*Valeriana officinalis* L	ناردين طبي	CAPRIFOLIACEAE	Rhizomes	1	0,15
Coriander	*Coriandrum sativum* L	قصبر	APIACEAE	Dried fruit	5	0,73
Flax seed	*Linum usitatissimum* L	زريعة الكتان	LINACEAE	Seeds	6	0,88
Cloves	*Eugenia caryophyllata* Thunb	القرنفل	MYRTACEAE	Flower buds	6	0,88
Ginger	*Zingiber officinale* Roscoe	الزنجبيل	ZINGIBERACEAE	Rhizomes	6	0,88
Rye	*Secale cereale* L	الشليم	POACEAE	Dried fruit	2	0,29
Lemongrass	*Cymbopogon citratus* (DC)Stapt	حشيشة الليمون	POACEAE	Leavers	2	0,29
Rosemary	*Rosmarinus officinalis* L	اكليل الجبل , أزير	LAMIACEAE	Leaves, Flowering tops	4	0,58
Basil	*Ocimum basilicum* L	حبق	LAMIACEAE	Leavers	2	0,29
Common sage	*Salvia officinalis* L	سالمة	LAMIACEAE	Leavers	1	0,15
Origan marjoram	*Origanum majorana* L	الزعتر	LAMIACEAE	Leavers	2	0,29
Saffron	*Crocus sativus* L	زعفران	IRIDACEAE	Styles and stigmas	1	0,15
Wheat	*Triticum aestivum* L	القمح	POACEAE	Wheat germ	1	0,15
Watercress radish	*Raphanus sativus* L	الفجل	BRASSICACEAE		1	0,15
Curcuma	*Curcuma longa* L	الكركم	ZINGIBERACEAE	Rhizomes	2	0,29
Fragrant mint	*Mentha suaveolens* Ehrh	دومران	LAMIACEAE	Leaves, Flowering tops	14	2,05
Amoide verticillata	*Ptychotis verticillata* Duby	النوخة	APIACEAE	Flowering tops	18	2,63
Nigella	*Nigella damascena* L	السانوج , الحبة السوداء	RANUNCULACEAE	Seeds	7	1,02
Chalep Street	*Ruta chalepensis* L	الفجلة	RUTACEAE	Aerial part	3	0,44
White Marrubus	*Marrubium vulgare* L	مريوت , مريوة , مروث	LAMIACEAE	Leaves, Flowering tops	2	0,29
Lavender	*Lavandula angustifolia* Miller	خزامى	LAMIACEAE	Leaves, Flowering tops	2	0,29
Ayapana	*Ayapana triplinervis* Spach		ASTERACEAE	Leaves	1	0,15
Cinnamon	*Cinnamomum sp*	القرفة	LAURACEAE	Barks	4	0,58
Poulliot mint	*Mentha pulegium* L	فليو	LAMIACEAE	Leaves, Flowering tops	12	1,75
Oxycedral juniper	*Juniperus oxycedrus* L	عرعار كاري , شربين	CUPRESSACEAE	Cade oil, Leaves	2	0,29

Green tea	*Camellia sinensis* L	الشاي الأخضر	THEACEAE	Young leaves and buds	4	0,58
Myrtle	*Myrtus communis* L	ريحان	MYRTACEAE	Leaves	3	0,44
Mallow	*Malva sylvestris* L	الخبيزة	MALVACEAE	Flower	3	0,44
Parsley	*Petroselinum sativum* Hofm	معدنوس	APIACEAE	Dried fruit	1	0,15
Pepper mint	*Mentha piperata* L	نعناع الفطور	LAMIACEAE	Leaves	4	0.58
Eucalyptus	*Eucalyptus globulus* Labill	كاليتوس	MYRTACEAE	Aged leaves	1	0,15
Grenadier	*Punica granatum* L	رمان	PUNICACEAE	Fruit peels	3	0,44
Packaged herbal teas					33	4,82
Did not respond					6	0,88

4. Conclusion

At the end of this study, we were able to identify the different plants used and obtain detailed information on the composition and mode of administration of the herbal teas. Although our survey is only half virtual, our survey opens the door to further research in the future, and the perspectives of this study are as follows

- To deepen the analysis of the therapeutic efficacy of medicinal plants, especially in terms of toxicology.
- To carry out more in-depth studies on all the medicinal plants reported in order to isolate and identify the active principles using more precise methods such as: HPLC and NMR.

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Author Contribution

The study and design were conceptualized and designed by ML and HA. The first draft of the manuscript was written collaboratively by ML, HA, and DHMK. The work was revised critically by HA. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declared no conflict of interest.

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Review article
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Current Perspectives on Medicinal and Aromatic Plants

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A Comparative Study on Conventional and Advance Techniques for Plant Extraction and Effect on the Extract Yield

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Abstract

Medicinal plants are bringing so much attention from the researchers, nutritionists and consumers due to their potent nutritional value and ethno-medicinal properties. Extraction plays a critical role to study the medicinal properties of plants, herbs and spices. The present review was conducted to elaborate the advance method of extraction with small use of solvent and better yields of extract in short time duration. Nowadays purity is a major concern for the researcher to evaluate effective analysis. To obtain the phytochemicals from the plant majorly depends on the extraction procedure. Inappropriate extraction may cause loss in quantitative phyto-components. Advance methods like Microwave Assisted Extraction (MAE), Accelerated Solvent Extraction (ASE), Solvent Phase Extraction (SPE) and Ultrasound Assisted Extraction (UAE) are studied on compare with conventional methods on the basis of yield, efficacy and purity of extracts. In this review, advance extraction techniques have briefly discuss based on their strength to obtain high yields and better purity of extract to assess the economical attainability of these methods and further used in chemical, biological and pharmaceutical analysis.

Keywords: Accelerated solvent extraction, Conventional methods, Medicinal plants, Soxhlet extraction, Ultrasound assisted extraction

1. Introduction

Medicinal plants are naturally obtained plant products which are used since ancient era to cure the specific diseases (Goswami and Chatterjee, 2014). Being a large source of therapeutic phytochemicals, medicinal plants are strongly in demand in pharmaceutical industries to the development of novel drugs (Venugopal and Liu, 2012). The urge to use natural products in the skin benefits as an alternative to conventional medications and synthetic products is leading an interest in medicinal plant research and industrial purpose for their formulation (Mukherjee et al., 2011). The purpose of extracting phenolic compounds from their plant sources is to liberate them from the vascular structures where they are found, either through rupturing plant tissue or through a diffusion process (Crozier, 2003). Nowadays consumers preferably demanding natural ingredients based products instead of artificial one because organic products are safe, reliable and eco-friendly. Manufacturers are incorporating green and organic compounds in their products to attain the

attention of consumers. Natural compounds are progressively searched by researchers to replace the synthesized compounds. Plants are the major sources of natural compounds on earth. Alkaloids, phenols, flavonoids, essential oil and polysaccharides are the active compounds that plants produce depends on their environmental growth. Such bioactive compounds have been used in the pharmaceutical, nutraceutical, cosmetic, and food processing and pesticide industries (Mykhailenko et al., 2019; Hosseini et al., 2018; Mohd-Setapar, 2018; Saucedo-Pompa et al., 2018; Nandhini et al., 2013).

To obtain bioactive constituents from plant materials conventional method of extraction are based on Solid Liquid Extraction (SLE) technique. Maceration, soxhlet extraction and hydro-distillation are some commonly used extraction methods. Although conventional methods of extraction are reliable to use, they are frequently under criticize for excess amount of solvent consumption and take a time for the extraction (Idham et al.,2017). Extracts obtained by different extraction procedures probably possess different composition and properties (Abuduwaili et al., 2019).

Therefore, to obtain desired extract, extraction techniques must be chosen carefully. Optimization of extraction parameters should be conducted to enhance extraction efficacy, reduce solvent and energy consumption (Chemat et al., 2019; Perino and Chemat, 2019). Afterwards, plant by products from food processing unit used as a starting materials might be helpful to enhance the sustainability in extraction process (Barreira et al., 2019; Andrade et al., 2019).

This review emphasized the comparative study in conventional and recent advancement in numerous extraction techniques acquire bioactive components from plant and effect in the extract yield.

2. Pre-treatment method of plants

2.1 Grounding of sample

Before extraction, pre-treatment methods are used to prepare samples for the extraction by washing, drying and grinding of the plant material. To obtain active constituents from plant materials, extraction often involves soak the material in solvent for a certain time period. Now, extracts are kept for further processing. Extraction involves separation of medicinally active part of plants using selective solvents through standard methods (Handa, 2008). The main aim of extraction is to separate the soluble plant metabolites, leaving behind the insoluble residue. To make the extraction efficient, solvent must have contact with the target analytes and particle size 0.5 mm is ideal for efficient extraction (MOA, 2013).

2.2 Drying methods of plant

Sample takes usually 3-4 months for air drying, and it depends on which part of plant are used fruits, leaves and bark. In the air drying method, heat labile components of plant are maintained. However, this method takes long time to dry in comparison to other methods and is more vulnerable to contamination at unstable temperature condition. Oven-drying method consumes thermal energy to get rid of moisture content from the sample. This method is considered as finest and fast thermal process for preserving phytochemicals. The maximum antioxidant activity was reported in *Cosmos caudatus* after oven-drying at 44.5 °C upto 4 hours using 80% methanol, and in optimum 80% methanol extracts at 44.12 °C (Mediani et al., 2013). This method takes a short time period to complete the extraction. Oven drying at 65 °C temperature leads to increase in total phenolic content of sage plants (0.66 mg of GAE/g of DM) (Hamrouni-Sellami et al., 2013).

Microwave drying technique uses electromagnetic radiation. The electric field generates simultaneous heating via dipolar

rotation; alignment towards the electric field of the molecules exhibiting an induced dipole moment which creates oscillation of the molecules (Kaufman and Christen, 2002). Oscillations vigorously generate collision between molecules and resulting in rapid heating of the samples. This drying method can take lesser time but may be caused damage in phytoconstituent present in the sample.

Freeze drying method is based on the sublimation process. Plant samples are freeze-dried (lyophilized) for analysis. There is a presumption that freeze-drying is better to other preservation techniques and effectively retains the therapeutic properties of plants (Abascal et al., 2005).

2.3 Extraction methods

Extraction is the separation of medicinally active components using various solvents from the plant sample followed by different procedures (Handa, 2008). Extraction method should be reliable, cost effective, environmentally friendly and with high yield value. The objective of extraction methods is to separate out the active components leaving behind the residue. Various extraction methods for the plants are presented in Fig. 1 and discussed below.

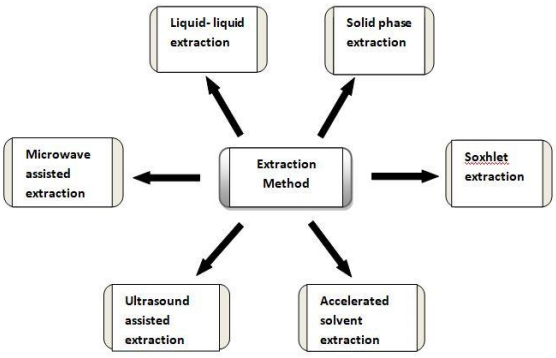

Figure 1. Extraction methods for plants

2.3.1 Liquid-liquid extraction

This method is particularly used to extract two immiscible liquid on the basis of their respective solubility presented in Fig. 2. Opium alkaloid was extracted using liquid–liquid extraction process (Yoshimatsu and Shimomura, 1992) with a small modification. Capsule powder sample (ca. 50 mg) was extracted with 5 ml acetic acid (5%) via sonication and further allows mixing in vortex mixer. Then, filter the sample and 3 ml of it washed with chloroform (3 ml). Using 28% ammonia, the aqueous phase made alkaline and extracted with chloroform and isopropanol mixture (3:1) up to three times (2ml, 1ml and 1 ml). The organic-base extract was concentrated to dry under nitrogen stream, and the residue obtained was dissolved in an accurate volume of 50% methanol and examined by HPLC. The chloroform solvent phase employed to wash 5% acetic acid extract, was recover, concentrated and dissolved in an appropriate volume of 50% methanol and similarly can be evaluated by HPLC.

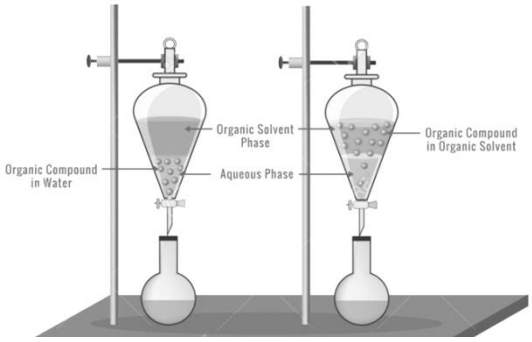

Figure 2. Liquid-liquid extraction

2.3.2 Solid-phase extraction (SPE)

SPE is a fast, selective procedure commonly used in analytical labs for the analyte extraction. Schematic diagram for SPE extraction in fig 3. A reversed-phase solid-phase extraction was used for SPE extraction. A plant sample (ca. 50 mg) was extracted with 5 ml of solvent (5% acetic acid, 0.1 M sodium citrate buffer of pH 6.0, water) and allowed to sonicate for 30 min. After mixing in vortex mixer (1 min) and centrifugation (18000g, 10 min), 3 ml of the supernatant was purified by SPE extraction cartridge according to the protocols (Waters, 2001). In

the detailed, SPE extraction cartridges were procured in methanol (3 ml) and then equilibrated in water (3 ml). In solid-phase extraction cartridge, 3 ml of crude extract was loaded and then cartridge washed with washing solvent (3 ml). The alkaloids were eluted with elution solvent twice (E1, 2 ml; E2, 1 ml) and each elute was concentrated to dry in nitrogen stream, dissolved in volume of 50% methanol and estimated by HPLC (Yoshimatsu, 2005).

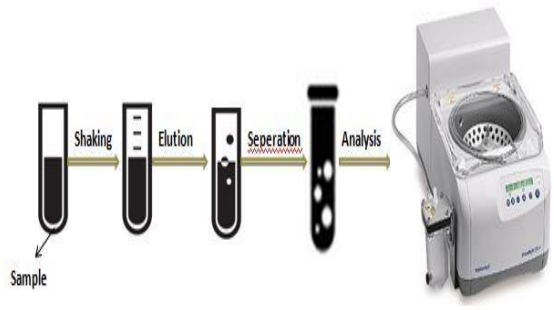

Figure 3. SPE extraction process

2.3.3 Maceration, infusion, decoction, percolation & soxhlet extraction

In maceration, coarse powdered plant sample is kept with the desired solvent in a stopper container for a definite time period and frequent stirring till the soluble component is dissolved. This is the best method to heat sensitive components present in plants (Anonymus, 1966). Infusion and decoction method follows the theory similar to maceration; both are allowed to immerse in the cold or boiled water. Boiling process might be damage heat sensitive component present in the extract. However, the sample is boiled in a certain amount of water for a specific amount of time for decoction, while the maceration process for infusion process is shorter [16]. In percolation method, a unique instrument percolator is used. Dry powder sample added with boiling water is enclosed in percolator and allow to the macerate up to 2 hours. In order to get concentrated extract, the percolation method is often carried out at a moderate rate (e.g. 6 drops/min) till the extraction is complete (Rathi et al., 2006). In soxhlet extraction, powdered sample is taken in thimble and solvent used for extraction is heated in the bottom flask, vaporizes into sample thimble, condenser to condense and drip back. When the liquid content reaches to siphon arm, it evacuated into bottom flask again and process continued. This extraction method required a small amount of solvent (Handa, 2008).

2.3.4 Microwave assisted extraction (MAE)

This method of extraction used electromagnetic radiations that carry plenty of energy presented in Fig 4. Plant sample in microwaves system are heated themselves through conventional heating method as opposed to heating from outside. Microwave heating may damage the plant cell wall and liberate active components for effective extraction. Previous studies reported that MAE method provides better yields of extracts and significantly higher than the conventional method for the extraction of active components (Citadin et al., 2016; Li et al., 2011; Patil and Akamanchi, 2017). The active compounds from *Eucalyptus globulus* have been extracted using MAE, UAE and EAE methods and it was observed that similar components obtained from the extracts. After testing the above-mentioned extraction methods the researchers suggested MAE methods because it has significant lower specific energy consumption (Gullon et al., 2019). MAE is the fastest method and consumes less solvent compared to UAE, SFE and maceration methods of extraction for active compounds. MAE extraction method gives a satisfactory extraction yield (Odabas and Koca, 2016).Aqueous alcohol used as a solvent in MAE extraction, provide an appropriate solvent polarity to extract the bioactive components from lime peel (Rodsamran and Sothornvit, 2019). Triterpene from *Centella asiatica* extracted using MAE demonstrated an increasing yield twice of soxhlet extraction and absolute alcohol is used as a solvent at 75˚C and

irradiation power consumption of 600 W (Puttarak and Panichayupakaranant, 2013). In MAE, *Dioscorea hisptida* yields were highest using 85% ethanol at sample solvent ratios (1:12.5) (Kumoro and Hartati, 2015).

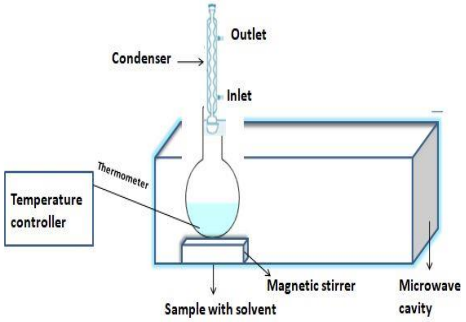

Figure 4. MAE extraction method

2.3.5 Ultrasound- assisted extraction (UAE)

UAE method use cavitations caused by ultrasound range 20 kHz to 2000 kHz and solvents to increase the extraction of active compounds (Handa, 2008). Schematic diagram for UAE extraction process presented in Fig. 5. The mechanic effect of ultrasound increases the surface contact between sample and solvent. The active phytochemicals may affect through formation of free radicals in more than 20 kHz of ultrasound. UAE is the best extraction method than other conventional methods to extract the *Elsholtzia ciliate* (Pudziuvelyte et al., 2018).

In order to shorten the extraction time and restrict exposure to high temperatures, UAE method was used in the extraction of thermolabile components, such as anthocyanin, from flower parts (Dhanani et al., 2017). *Withania somnifera* possess maximum extract yield in aqueous solvent (11.85%), compare to ethanol and aqueous ethanol solvent using UAE extraction method. It is reported that UAE extraction provide prominent extract yield as compared to MAE and SFE (Odabas and Koca, 2016; Kozioł et al., 2019; Rodsamran and Sothornvit, 2019).

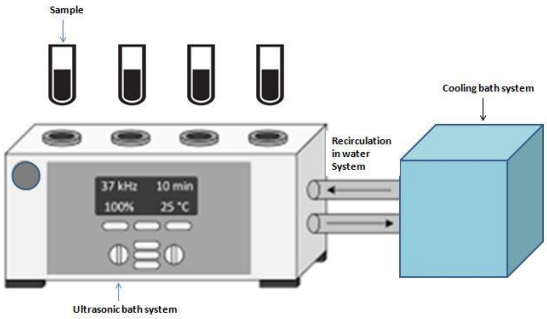

Figure 5. UAE extraction process

2.3.6 Accelerated solvent extraction

Accelerated solvent extraction process is an alternative of traditional method of extraction shown in Fig. 6. ASE takes advantages of magnified solubility that arise with increase in temperature of liquid solvent. On increasing temperature, viscosity of solvent decreased which allows easiest pass out of solvent matrix. Furthermore, analyte diffusion from sample matrix into solvent has been increased. In this way ASE method, gives similar yield of extract as obtained by solvent based extraction technique. Small consumption of solvent and short extraction time made this technique more efficient to use (Luthria et al., 2019). ASE method was observed the best for extraction of polycyclic aromatic hydrocarbons (PAH) (Wang et al., 2007).

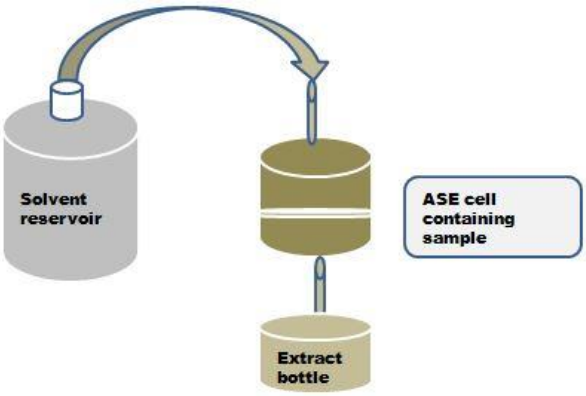

Figure 6. ASE extraction process

3. Discussion

Study on medicinal plants is the traditional approach to researchers and scientists via extraction of plants to analyze the chemical

components and further used in medication and ailment purpose. Nowadays various extraction techniques are in use than the conventional methods due to lack of time and solvent consumption in the extraction. Some extraction process of plant material is presented in Table 1.

Table 1. Different methods of extraction from plant material

Plant material	Analyte	Extraction method	Reference
Centella asiatica	Triterpene extract	Absolute alcohol solvent used for MAE	[33]
Dioscorea hisptida	Extract	Ethanol used as solvent with MAE	[34]
Elsholtzia ciliate	Plant extract	UAE	[35]
Flower part	Thermolabile component	UAE	[36]
Withania somnifera	Plant extract	Aqueous solvent using UAE	[37]
Plant part	Sample matrix	ASE	[41]
Plant part	polycyclic aromatic hydrocarbons	ASE	[42]
Plant part	Extraction of alkanes and LCOHs	ASE	[46]

This article demonstrated the comparable study of conventional methods with advance techniques is presented in Table 2.

Table 2. A comparative study on extraction process and their yield

Plant material	Extract	Extraction Process	Extract yield	Ref.
Vernonia cinerea	Leaves extract	SE	10.01%	[44]
Sapindus mukkorosi	Oil extract	MAE SE	40.12% 40.63%	[45]
Vegetable (celery, lettuce)	Extraction –recovery	QuEChERS ASE	70.2-133.5% 70.1-118.6%	[46]
Oleuropein	Plant extract	SE SFE	3.78% 1.43%	[49]
Cannabis sativa	Plant extract	SPE SFE	91.1% 36.18-37.85%	[50]

Since ancient time, plants are best alternative for the ailment purpose has no adverse reaction in the health. Biological efficacy is widely depends on the phytochemicals observed in the plant extract. Extract yield can varies with primary process of extract preparation, solvent ratio and parts of plant used for the extraction. Soxhlet extraction method is one of the traditional procedure for the extraction. Soxhlet extraction of *Vernonia cinerea* leaves provide yield of (10.01±0.85% w/w) of extract (Alara et al. ,2018). The previous study depicts that essential oil yield in *Sapindus mukkorosi* was 40.12% and 40.63% via MAE and SE methods, respectively (Hu et al., 2021).

The extraction of pharmaceuticals from plants was done by QuEChERS method was comparable to ASE method. QuEChERS method performed in short time with less amount of solvent is used. This method is quite efficient to obtain pharmaceuticals in vegetables and safe for the agricultural produce used by humans (Chuang et al., 2015). A single step ASE method was efficient in extraction of alkanes and LCOH's from plants using less amount of solvent in significant time (Carnahan et al., 2013). The soxhlet method of extraction was effective to extract phenolic compounds from plants. MAE and UAE extraction techniques are found good to extract antibacterial components from plant material (Kothari et al., 2012).

Oleuropein yield was found in soxhlet extraction method was 37.8 mg/g dried leaf whereas, the better yield was observed in SFE method was 14.26 mg/g dried leaf (Sahin et al., 2011). By using a single SPE step, a final fraction with 91.1% of THC was obtained and in SFE, the highest content was 37.85% and 36.18% at 33 MPa and 15 MPa, respectively (Gallo-Molina et al., 2019). Literature survey revealed that tetrahydrocannabinol (THC) from *Cannabis sativa* L. using solid phase extraction process yields superior amount of extract. ASE method is the efficient way to extract vegetable (celery, lettuce) with prominent extract yield.

Conclusion

Extraction plays a key role to study the presence of phytochemicals in herbal plants. Pre-extraction process like drying and grinding eventually have an effect on the chemical constituent of sample and finally in the extract of plant sample. This study concluded that each method of extraction has their significant results either in terms of yield or the solvent required for the extraction. Soxhlet method is the traditional method having a good yield of extract consumes a lot of solvent and gives purity of extract. UAE, SPE, SFE and ASE methods are quite promising with respect to solvent consumption and yield of extract. Overall study depicts that ASE and SPE method could be better suggest for the extraction on the basis of efficiency, quality, purity, reproducibility, economically and yield efficacy of extract. The new bioactive components will come in force continually to explore ingenious extraction methods to get meritorious extract yield from the whole plant.

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Author contribution

Dr. Nisha Mehra involved in design, conceptualize, data curation, writing, review, editing and drafting the manuscript.

Conflicts of interest

The author declares no potential conflict of interest.

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Herbal Remedies Used for the Treatment of Infertility in Females by Traditional Tealers in the Northwest of Algeria

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Abstract

Ethnopharmacological relevance: According to the world health organization, infertility was a global health issue affecting millions of people of reproductive age worldwide. Available data suggests that between 48 million couples and 186 million individuals have infertility globally.

Aim of the study: The aims of this work to inventory plants used in the treatment of female infertility by the herbal healers in the northwest of Algeria.

Materials and methods: A number of 30 herbal healers and resource persons were interviewed. This ethnobotanical survey allowed to identify 41 recipes in which involved 49 plant species belonging to 23 family used to treat infertility in women by traditional healers in the northwest of Algeria.The scientific name, family, common name, part used, mode of preparation as well as of administration, days of treatment and the indication are provided.

Results: Results show that The Lamiaceae family is the best represented with 9 species and 32.14% of the frequency of use, followed by the Apiaceae with 8 species and Asteraceae with 4 species and frequency of use 9.52% for each .The aerial parts are the most commonly parts used with a rate of 22% of total parts of the plant, followed by the leaves with 18%, seeds 16%,root and rhizome 12%,fruit and flower (10% each); bark bulb(4% each), the kernel and pollen grains(2% each) Infusion is the main mode of preparation with rate of 40% of total preparations, mixture with 37%, decoction with 17%, and finally paste with 6%. Recipes were principally taken orally (83 %) or topically (17%) as a poultice (10%) or as a vaginal steaming (7%).

Conclusions: Herbal healers in northwest of Algeria have a wide range of herbal remedies used to treat infertility in females; their knowledge is a natural heritage from their ancestors transmitted through centuries.

Key Words: ethnobotany, infertility, recipes, herbal healers, northwest of Algeria.

1. Introduction

Plants and natural products have played a significant role in curing and preventing a variety of diseases occurring in humans and animals, and continue to provide new bioactive leads for researchers (Singh et al., 2020). In this sense, ethnobotany can provide the tools for documenting this knowledge (Katiyar et al., 2012).

Ethnobotany is defined as the study of the dynamic relationship between plants and people. It draws on a range of disciplines, including natural and social sciences, to show how conservation of plants and of local knowledge about them can be achieved. This great wealth of knowledge has been acquired through direct personal experience and is passed on from generation to generation mainly through oral testimonies .it is considered as the starting point for plant-based new drug discovery since it reduces the number of plants candidates for the research, therefore, this integrated approach would lead to saving of cost and time, coupled with enhanced success rate (Signorini, 2007; Voeks, 2017).

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse; it affects millions of people of reproductive age worldwide. Estimates suggest that between 48 million couples and 186 million individuals live with infertility globally (WHO, 2020). Our work consists in making an inventory of the plants used in the treatment of infertility by the herbal healers in the north-west of Algeria.

2. Material and Methods

2.1. Presentation of the Study Region

Algeria is the cradle of successive ancient civilizations which have brought vast knowledge on the use of plants whether for culinary or therapeutic purposes; also its diversity of geography, climate and soil type create the perfect environment for several and different plants that make Algeria has a great green fortune in the plant world. The research program was based on visits to the herbalists and healers in three neighboring states in the north-west of Algeria: Ain Temouchent, Mascara and Sidi Bel Abbes. Their total area is 17470 km^2 .They are an integral part of the Tell region, which means the Mediterranean coastal zone, which is bordered by mountain ranges.

1. Aïn Témouchent is bounded by three cities, to the east by Oran, to the southeast by Sidi-Bel-Abbès, to the south-west by t Tlemcen, and to the north-west by the Mediterranean Sea that borders it for a distance of about 80 km. It has a hot Mediterranean climate with dry summer. The average temperature in Aïn Temouchent is 19.1 °C and the precipitation averages 316.2 mm.

2. Mascara is delimited to the north, by t Oran and Mostaganem; to the east, by Tiaret and Relizane; to the south, by Saïda; to the west, by Sidi Bel Abbès.The climate of the city is Mediterranean with a tendency to semi aridity.

3. Sidi Bel Abbès is located in the northwest of the country, it is geographically limited as follows: To the north by Oran; In the North-West by Ain Témouchent; In the North-East by Mascara; To the west by Tlemcen; To the east by Mascara and Saïda; In the South by Nâama and El Bayadh; In the South-East by of Saïda. Sidi-Bel-Abbès has a hot Mediterranean climate with dry summer. The average annual temperature in is 18.9 ° C and the average rainfall is 337.4 mm. Data collection from herbal healers: The data was collected using questionnaire sheets through an ethnobotanical survey based on interviews with people in contact with plants throughout the study region. Five towns were selected at random from the study area in the four states (Mohammadia and Sig in Mascara,

Alamria and Hamam Bohdjer in Ain Temouchent , the city center of Sidi Bel Bbbes). The people surveyed are traditional healers and resource persons recognized by society as having a good knowledge of medicinal plants. Several interviewed are illiterate. The study was carried out during 7 months, from December 2020 to June 2021.The number of people questioned is 30, between 19 and 70 years old, split between both sexes (1/3 female, 2/3 male).the information collected are related to geographic data , the age and the experiences of the interviewee, the species used in the treatment of the female primary infertility , the method of preparation ,the used parts of the plant , composition of the recipe as well as its mode and time of use and its indication.

3. Results and Discussion

The analysis of the results of the survey on medicinal plants used in the treatment of primary infertility by the herbal healers in three states of the Algerian Tell Ain Temouchent, Mascara and Sidi Bel Abbes, brings out a number of 41 recipes which make use of 49 inventoried species, spread over 23 families (figure 1), including in addition to scientific name and families of plants, the vernacular name in English and Arabic, the method of preparation, the way and the days of administration of the remedy and its indication (Table1).

The Lamiaceae family is the best represented with 9 species and 32.14% of the frequency of use, followed by the Apiaceae with 8 species and Asteraceae with 4 species and frequency of use 9.52% each; Brassicaceae with 4 species (8.33%), then Rosaceae with 3 species (3.57). Amaryllidaceae, Thymelaceae and Amaranthaceae with 2 species for each (3.57;4.76;3,57 respectively). While the rest of the 15 families represented together by 15 species and a rate of 25% in their total frequency of use (Fig. 1).

The predominance of an organ uses over another, in the therapeutic field, arises from their concentration of active ingredients. It was thus found that the areal parts are the most used with a rate of 22%; followed by the leaves 18%, the seeds (16%), the roots and rhizomes (12%), fruit and flower (10% each); bark bulb(4%each), kernel and pollen grains (2% each) (Figure 3).

Infusion is the main mode used, entering into the preparation of 43.90% of recipes, followed by mixture (34.14%) decoction (17.07%), and finally as a paste (4.87) (Figure 2).

In our study, the oral route is the main mode adopted with a rate of 83% while the topical route represents 17% poultice (10%) and as vaginal steaming (7 %) (Figure 5). Finally, in only 39% of the recipes, plants are used alone. In 61% of the recipes, it is a combination of at least two plants or a mixture with other ingredients like milk, honey, olive oil, propolis (Figure 4). The herbal healers in the northwest of Algeria have confirmed that the recipe they provide were used by women who had difficulty in conceiving that for some women lasted more than 8 years without result from the conventional medicine, and have succeeded to conceive after trying traditional medicine.

Figure 1. Map showing all the surveyed areas

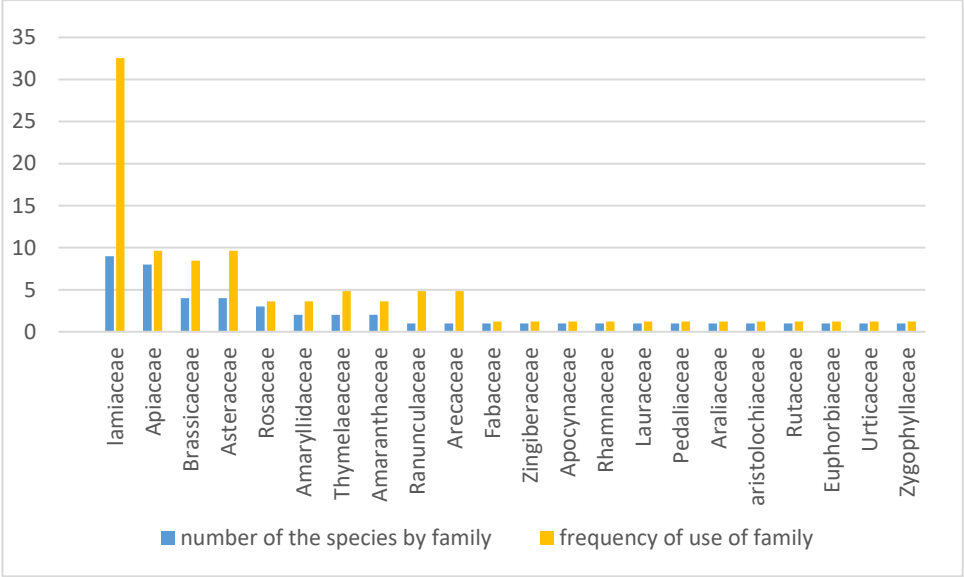

Figure 2. Number of species by family and the frequency of use of families

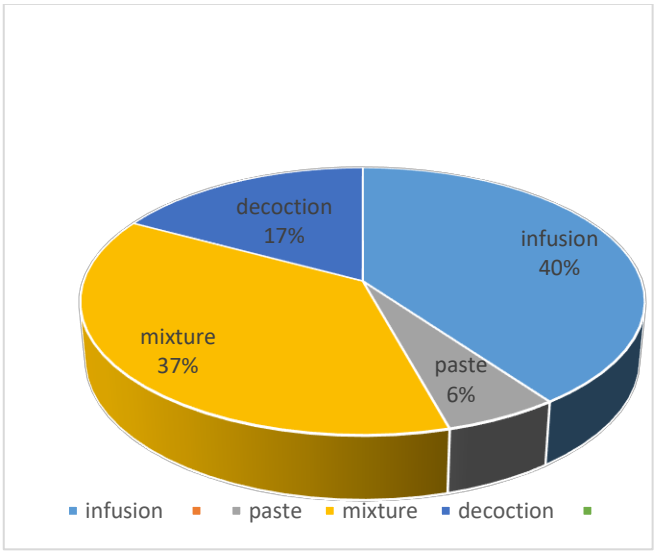

Figure 3. Modes of preparation of anti-infertility herbal recipes

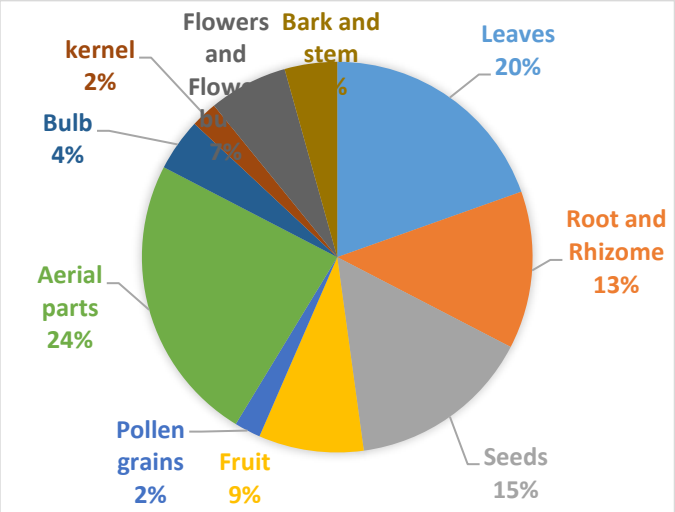

Figure 4. Parts used in anti-infertility herbal recipes

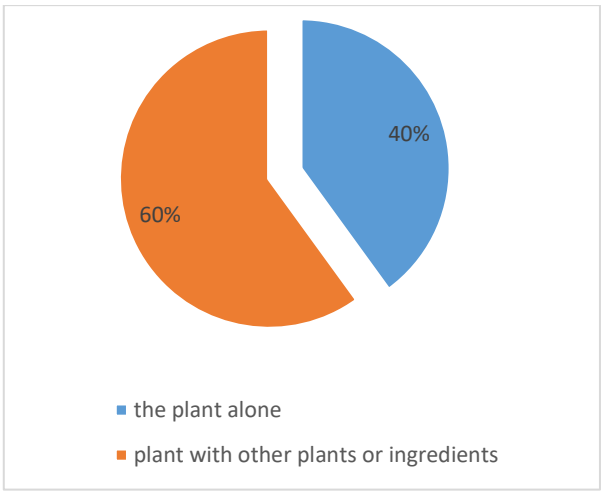

Figure 5. Number of ingredients in recipe

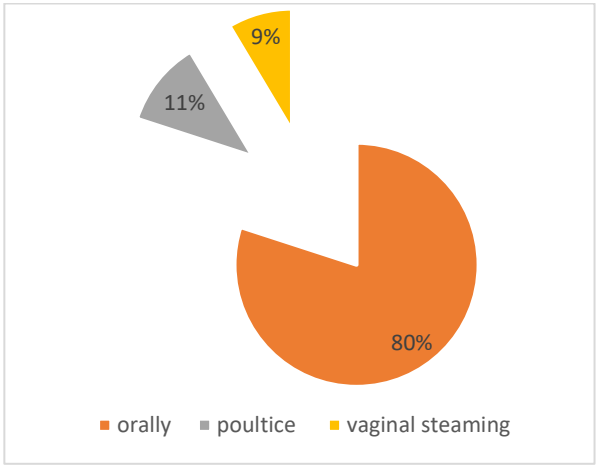

Figure 6. Roots of administration of the recipes

Table 1 List of recipes used by herbal healers in the treatment of primary infertility in the north west of Algeria

Scientific name	Family	Common name Arabic-English	Used parts	Mode of preparation	days /dose of administration	Indication according to the herbalist
1/ -Urtica dioica	Urticaceae	الحريق -stinging nettles	Leaves	Infusion	One cup everyday	Ovulation inducer Dietary supplement
2/ -Nigella sativa -Lepidium sativum -Phoenix dactylifera	Ranunculaceae Brassicaceae Arecaceae	الحبة السوداء-Black Cumin حب الرشاد -Garden cress التمر-date palm	Seeds Seeds Fruits	fruits to be filled with a mixture of equal amounts of both seeds	To be taken early morning once daily from the 1st to the 7th day of the cycle.	Dietary supplement
3/ -Phoenix dactylifera	Arecaceae	نخلpalm	Palm pollen	To be mixed with honey and prepared as paste	One or two pills to be taken everyday	Ovulation inducer
4/ -Lavandula spica -Artemisia herba alba -Allium sativum	Lamiaceae Asteraceae Amaryllidaceae	الخزامىlavender الشيح- Mugwort الثوم –garlic	Aerial parts (flowers) Leaves Bulbs	Roast 2 cloves of garlic then add some of olive oil and smash, add 2 tablespoons of both leaves and flowers, to be used as vaginal poultice	To be used at night after 3 days of the end of the menstrual period	Vaginal infection
5/ -Lepidium sativum -Phoenix dactylifera	Brassicaceae Arecaceae	الرشاد -Garden cress التمر-date palm	Seeds Fruits	Prepare the decoction of the fruit, let it cool and then add some olive oil; mix until homogeneity	To be taken from the 1st to the 5th day of the cycle	Dietary supplement
6/ -Atriplex halimus	Amaranthaceae	القطف- Mediterranean saltbush	Aerial parts	Infusion	One cup be drunk twice a day during one month	Ovarian cysts
7/ -Raphanus sativus - Nigella sativa - Trigonella foenum-graecum	Brassicaceae Ranunculaceae Fabaceae	الفجل-radish الحبة السوداء-Black Cumin الحلبة-Fenugreek	Seeds Seeds Seeds	A mixture of one teaspoon of each seed's powder with a cup of honey	A tablespoon of the mixture twice every day with a cup of milk (optional)	Dietary supplement
8/ -Origanum majorana	Lamiaceae	البردقوشMarjoram	Leaves	Infusion	One cup every day from the 2nd to the 14th day of the cycle	Ovulation inducer
9/ -Zingiber officinale	Zingiberaceae	زنجبيل-Ginger	Rhizome	Infusion	One cup every day from the 1st to the 5th day of the cycle	Ovulation regulator

10/ *- Tabernaemontana crassa*	Apocynaceae	القطف Mediterranean saltbush	Fruits	A mixture of one teaspoon of the grated with a cup of milk and some honey	One cup every night before sleep during the menstrual period	Dietary supplement
11/ *- Saussurea costus*	Asteraceae	Root	A teaspoon of each to be prepared as infusion	Infusion	Three times a day	Hormonal disorders
12/ *- Salvia officinalis* *- Salvia rosmarinus* *- Foeniculum vulgare*	Lamiaceae Lamiaceae Apiaceae	تفاحة ادم-Adam's apple flower	Leaves Leaves Fruits	A warmed mixture of equal amounts of the bulb juice, olive oil, leaves and aerial parts as vaginal poultice	One cup after lunch and one cup after dinner	Hormonal disorders
13/ *-Salvia officinalis* *-Origanum majorana* *-Vitex agnus-castus*	Lamiaceae Lamiaceae Lamiaceae	القسط الهندي-costus	Leaves Leaves Leaves	Infusion	One cup a day from the 2nd to the 9th day of the cycle	Ovarian cysts
14/ *-Artemisia herba alba* *-Lavandula spica* *-Allium cepa*	Asteraceae Lamiaceae Amaryllidaceae	المريمية-sage إكليل الجبل-rosemary البسباس-Fennel	Leaves Aerial parts (flowers) Bulbs	A teaspoon of each to be prepared as infusion	To be used after 3 days of the end of the menstrual period	Vaginal infection
15/ *-Brassica rapa*	Brassicaceae	اللفت-turnip	Seeds	Infusion	One cup each morning during one month for the couple	Dietary supplement
16/ *-Lavandula stoechas* *-Nepeta cataria* *-Thymus vulgaris* *-Ziziphus jujuba*	Lamiaceae Lamiaceae Lamiaceae Rhamnaceae	حلحال- French Lavender النابطة-lesser calamint زعتر-thyme السدر- jujube	Flowers Aerial parts Aerial parts Leaves	A teaspoon of each to be prepared as infusion	Last day of the menstrual period	Dietary supplement
17/ *-Calendula officinalis* *-Nigella sativa* *-Aquilaria malaccensis* *-prunus persica* *-Haloxylon scoparium*	Asteraceae Ranunculaceae Thymelaeaceae Rosaceae Amaranthaceae	القطيفة-pot marigold الحبة السوداء-Black Cumin عود غريس- Eaglewood الخوخ-Peach الرمث-Saxaul	Flowers Seeds Stem wood Leaves Leaves	Infusion	One cup every night during 40 days	Ovarian cysts
18/ *-Salvia officinalis* *-Syzygium aromaticum*	Lamiaceae Myrtaceae Thymelaeaceae Lamiaceae	الميرمية-Sage قرنفل-Clove عود غريس- Eaglewood كف مريم--Chaste tree بردقوش- marjorum	Leaves Flower buds Stem wood Leaves Leaves	Infusion	One cup every night during 40 days	Menstrual cycle disorder Hormonal disorders

-Aquilaria malaccensis *-Vitex agnus-castus* *-Origanum majorana* *-Lavandula spica* *-Cinnamomum verum*	Lamiaceae Lamiaceae Lauraceae	الخزامى-Lavender القرفة-Cinnamon	Aerial parts (flowers) Barks			
19/ *-Sesamum indicum* *-Prunus dulcis*	Pedaliaceae Rosaceae	السمسم-Sesame اللوز-Almond	Seed Kernel	Equal amounts of seeds, crushed kernels, pollen to be mixed with honey and prepared as a paste	One spoon at morning during the ovulation days, for the couple	Dietary supplement
20/ *-Panax ginseng* *-Phoenix dactylifera*	Araliaceae Arecaceae	الجنسنج-Ginseng نخل-Palm	Roots Palm pollen	A mixture of the root's powder, palm pollen, propolis, crushed nuts and one cup of milk	One cup before the two principal meals during the ovulation days for the couple	Aphrodisiac Dietary supplement
21/ *-Calendula officinalis*	Asteraceae	القطيفة-Pot marigold	Flowers	Infusion	One cup to be taken from the 1st to the 7th day of the cycle	Ovarian cysts Hormonal disorders
22/ *-Nigella sativa* *-Aquilaria malaccensis* *-Calendula officinalis* *-Saussurea costus* *-Bunium mauritanicum* *-Aristolochia baetica*	Ranunculaceae Thymelaeaceae Asteraceae Asteraceae Apiaceae *Aristolochiaceae*	الحبة السوداء-Black Cumin عود غريس- Eaglewood القطيفة-pot marigold القسط الهندي-costus بكبوكة-تالغودة-banium برستم-Common Barberry	Seeds Stem wood Flowers Roots Roots Roots	A mixture of equal amounts of each plant and honey	One spoon to be taken on an empty stomach in the morning during one month	Uterine fibroids Hormonal disorders
23/ *–Vitex agnus-castus*	Lamiaceae	كف مريم-Chaste tree	Leaves	Infusion	One cup every day from the 2nd till the 9th day of the cycle	Hormonal disorders
24/ *-Daucus carota*	Apiaceae	كلخ – Wild carrot	Seeds	A mixture of the seed's powder with milk or honey	One spoon to be taken twice every day, in the early morning and at night before sleep	Emmenagogue Aphrodisiac
25/ *-Raphanus sativus*	Brassicaceae	الفجل- Radish	Seeds	Decoction	One cup to be taken in the early morning during one month	Ovulation stimulator

26/ -Pimpinella anisum	Apiaceae	حبة حلاوة-Aniseed	Fruits	Powder	One spoon to be taken on empty stomach every morning during one month	Ovarian failure
27 -Thymelaea hirsuta	Thymelaeaceae	مثنان -the Sparrow-worts	Aerial parts	Infusion	One cup to be taken twice a day, two hours before the principal meals. From the 2nd till the 7th day of the cycle	Urinary tract infection
28 - Ruta chalepensis	Rutaceae	فيجل-Fringed rue	Aerial parts	A mixture with whole wheat flour, salt, butter and water and prepared as bread	To be taken twice a day from the 4th till the 7th day of the cycle	Emmenagogue Painful menstruation
29 -Marrubium vulgare	Lamiaceae	مريوة مرة- horehound	Aerial parts	Decoction to be used as vaginal steaming	To be used the last day of the menstrual cycle	Uterine fibroids
30/ -Angelica archangelica	Apiaceae	حشيشة الملاك-Angelica	Roots	Decoction	One cup to be drunk in the early morning during Three months maximum	Menstruation disorders Painful menstruation
31 - Peganum harmala -Allium cepa -Thymus vulgaris -Lavandula spica	Zygophyllaceae. Amaryllidaceae Lamiaceae Lamiaceae	حرمل-Wild Rue البصل-onion زعتر-thyme الخزامى-lavender	Aerial parts Bulbs Aerial parts Aerial parts (flowers)	Decoction to be used as vaginal steaming	To be used the last day of the menstrual cycle	Uterine fibroids Ovarian cysts Uterine weakness
32/ -Marrubium vulgare -Thymus vulgaris	Lamiaceae Lamiaceae	مريوة مرة- Horehound زعتر-thyme	Aerial parts Aerial parts	An amount of the aerial parts to be mixed with preheated olive oil and used as vaginal poultice	At night during three days after the end of the menstrual cycle	Uterus cleansing
33/ -Petroselinum crispum	Apiaceae	بقدونس-Parsley	Aerial parts	A mixture of the pre-steamed plant with olive oil to be used as abdominal poultice	A day after the end of the menstrual cycle	Menstrual cycle disorders

34/ -Alchemilla vulgaris	Rosaceae	رجل الاسد- lady's mantle	Aerial parts	Decoction to be used as vaginal steaming	A day after the end of the menstrual cycle	Menstrual cycle disorders uterine fibroids
35/ -Euphorbia hirta	Euphorbiaceae	فربيون-Euphorbia	Aerial parts	Infusion	One cup a day during one month	The genitourinary tract infections
36/ *-Anastatica hierochuntica*	Brassicaceae	شجرة مريم-Jericho Rose	Flowers	Decoction	One cup every morning from the 3rd to the last day of menstruation	Ovulation stimulator
37/ *-Salvia officinalis -Anastatica hierochuntica*	Lamiaceae Brassicaceae	الميرمية-Sage شجرة مريم-Jericho Rose	Leaves Flowers	Infusion	One cup every day during the menstrual cycle	Ovarian cysts
38/ *-Salvia officinalis -Matricaria chamomilla -Origanum majorana*	Lamiaceae Asteraceae Lamiaceae	الميرمية-Sage بابونج-Flowers بردقوش- marjorum	Leaves Flowers Leaves	Infusion	One cup every day from the 2nd day of the cycle until the 8th day	Ovarian failure
39/ -Salvia officinalis -Origanum majorana -Atriplex halimus	Lamiaceae Lamiaceae Amaranthceae	الميرمية-Sage بردقوش- marjorum القطف- Mediterranean saltbush	Leaves Leaves Aerial parts	Infusion	One cup every day during one month	Ovarian cysts
40/ -Thymus vulgaris	Lamiaceae	زعتر-Thyme	Aerial parts	An amount of dry aerial parts ground and mixed with Couscous ingredients and cooked with	To be eaten the last days of menstrual cycle	Menstrual cycle disorders
41/ *-Cuminum cyminum -Coriandrum sativum*	Apiaceae Apiaceae	كمون-cumin قصبر-Coriander	Fruits Fruits	Infusion	One cup every day during one month	Ovarian cysts

All women were in the age of procreation and suffered from primary infertility that means they haven't achieved a pregnancy before (Who 2020). Polycystic ovary syndrome (PCOS) is the most common reproductive and metabolic disorder affecting women of reproductive age causing menstrual dysfunction and infertility (Guo et al., 2021). In our study, PCOS is the most common reason for consultation.

Some female herbal healers, generally old women use other therapies beside the herbal remedies as the cupping therapy on the left and right lumber of the abdomen to stimulate the ovaries or use a massage technique on the abdomen in specific days.

4. Discussion

The northwest of Africa, has a rich medicinal biological diversity that has been recorded. In Algeria, 16 species out of the 45 identified by this study had already been cited in similar studies (Hadj-Seyd et al., 2016). In comparison with other regional ethnopharmacological surveys about herbal

remedies used for the treatment of female infertility 29 species out of the 49 identified by this study had already been reported in a study made in Morocco. In both studies the 4 dominant families are Lamiaceae, Apiaceae, Asteraceae and Brassicaceae (Slighoua et al., 2019). The common species found in the ethnobotanical survey carried in Ghardaia are less than the one carried in Morocco even though Ghardaia is in the same country, this is due to geographical location, since the study regions of both studies are situated in the northern Africa. At the contrary, Ghardaia is a desert state with a totally different geography and climate.

Exclusively in our study, the specie *Atriplex halimus* is considered as very effective to remove the ovaries cysts from experience and herbal healers' testimonies. It is also notable the use of some toxic species; as euphorbia which is toxic at high doses (Slighoua et al., 2019). *Peganum Harmala*; which at high doses, it can produce paralysis (Lamchouri et al., 2012). *Aristolochia baetica* , *Origanum majorana* and *Pimpinella anisum* that can cause digestive disorders. *Marrubium vulgare* which is associated with dermatological and respiratory disorders; also *Foeniculum vulgare* which can cause ophthalmic disorders (Chaachouay et al., 2021). Sage and oregano are two medicinal herbs that have been traditionally used to treat female infertility. While further research is needed to confirm their efficacy and safety, some studies have suggested that these herbs may play a role in the regulation of FSH and LH hormones. FSH (follicle-stimulating hormone) is a hormone produced by the pituitary gland that stimulates the growth and development of follicles in the ovaries. LH (luteinizing hormone) is also produced by the pituitary gland and plays a role in ovulation and the production of estrogen and progesterone. (Sharma, 2016)

In vitro studies have shown that sage can increase the production of FSH and LH by the pituitary gland. A clinical study also showed that taking sage for 12 weeks increased FSH and LH levels in women with low levels of these hormones. *In vitro* studies have shown that oregano can inhibit the production of FSH by the pituitary gland. A clinical study also showed that taking oregano for 12 weeks reduced FSH levels in women with high levels of this hormone. (Najem et al., 2019).

The route of administration plays an important role, in the case of *Marrubium vulgare* and *Peganum hamala* is used as vaginal steaming or as vaginal poultice which reduces the risk of toxicity but in other species which are taken orally the risk is higher. Another example external use of lavender is a natural and effective way to treat vaginal infections. Evaporation and poultice are two simple and easy-to-perform method Lavender is a medicinal plant with many properties, including antibacterial, antifungal, and anti-inflammatory properties has been demonstrated by several studies. One study showed that the evaporation of lavender essential oil was as effective as antibiotic treatment for treating bacterial vaginosis. Another study showed that the application of a lavender poultice was as effective as antifungal treatment for treating vaginal candidiasis. These properties make it a potential natural remedy for the treatment of vaginal infections. *Ruta graveolens* is a plant that has been used traditionally for a variety of medicinal purposes. There is some evidence that certain species of *Ruta* may have some effect on ovarian insufficiency. For example, one study found that *Ruta chalepensis* may help to improve ovarian function in women with polycystic ovary syndrome (PCOS), a condition that can cause infertility. Additionally, there have been reports of women with ovarian insufficiency who have conceived after taking *Ruta* graveolens in our survey.

However, more research is needed to confirm the efficacy and safety of *Ruta* species for the treatment of ovarian insufficiency. In addition, many species of *Ruta* are considered to be toxic, so it is important to use them with caution. (Sharma et al., 2013). Further research is needed to confirm the efficacy and safety of this plants for the treatment of women infertilities caused by several mechanism like the regulation of FSH and LH hormones, polycystic syndrome or infections also. However, these medicinal herbs appear to have the potential to help treat female infertility.

5. Conclusion

Herbal healers in northwest of Algeria have a wide range of herbal remedies used to treat primary infertility in females; their knowledge is a natural heritage from their ancestors transmitted through centuries.

Therefore, this heritage deserves to be studied and used as a scientific base by researchers in order to determine their efficacy and safety and integrate it in the global health system so that people will have more benefits from the therapeutic virtues and active principles contained in these plants.

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Author Contribution

Dear authors please indicate the contribution of the authors. Cambria, 12 point, single line space, justified on both sides.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Effect of Extraction Solvents on Polyphenol Content and Antioxidant Activity of Carob Tree (*Ceratonia siliqua* L.) Leaves

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Abstract

Carob tree (*Ceratonia siliqua* L.) is widely used in traditional medicine and food. The aim of this study was to determine the most appropriate extraction solvent to optimize the extraction of polyphenols and flavonoids from the leaves, in order to obtain the highest antioxidant activity. In this context, the effects of six different solvents (100% methanol, 100% ethanol, 100% acetone, 50% (v/v) aqueous methanol, 50% (v/v) aqueous ethanol and 50% (v/v) aqueous acetone) with different polarities on the polyphenol content and antioxidant activity of carob tree leaf extracts were investigated. The total polyphenol and total flavonoid contents, as well as the antioxidant activity determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method of the extracts were evaluated. The results showed that carob tree leaves extracted with 100% methanol presented the highest values in terms of total polyphenol content (11.33± 0.08 mg EAG/g DM), total flavonoid content (8.55± 0.34 mg EC/g DM) and DPPH inhibition percentage (79.29 ± 3.75%). High positive correlations between total polyphenols, total flavonoids and antioxidant activity of carob tree leaf extracts were also observed. These results indicate that carob tree leaf extract obtained using an appropriate extraction solvent was able to enhance the protective effect against oxidative damage associated with free radicals.

Key Words: Antioxidants, Polyphenols, Flavonoïds, Extraction solvents, *Ceratonia siliqua* L. , Carob tree leaves.

1. Introduction

Carob tree (*Ceratonia siliqua* L.) is a typical Mediterranean species belonging to the Fabaceae family (Benmahioul et al., 2011). The fruit is a pod traditionally used as an antidiarrheal and in gastric ulcer, its seeds are natural appetite suppressants, and reduced to powder, they are used as an inexpensive alternative to cocoa in food, which is the reason why this tree is cultivated in its native countries. On the other hand, leaves have also pharmacological interest through their phenolic compounds content as: gallic acid, kaempferol, tannic acid, quercetin, rutin, biocanine, myricetin,

naringenin, genistein, catechin, taxifolin, epigallocatechin-3-gallate and epicatechin-3-gallate (Ouhaddou et al., 2014; Durazzo et al., 2014). Therefore, it is important to highlight the polyphenols and flavonoids as well as the antioxydant activity of carob tree leaves.

The solvent used for extraction is one of the most important factors influencing the efficiency of polyphenol extraction and the associated health benefits (Ngo et al. 2017). Polyphenols are often extracted from plant materials using polar solvents. Organic solvents such as methanol, ethanol and acetone, or aqueous mixtures, are generally preferred. Methanol is considered as a good solvent for extracting low-molecular-weight polyphenols, while aqueous acetone is more effective for extracting high-molecular-weight flavanols. Ethanol is also considered as a good solvent for polyphenol extraction overall (Do et al. 2014). The chemical composition of plant material varies from species to others. That's why, it is very difficult to propose a suitable extraction solvent for each plant material (Wijekoon et al. 2011).

Previous studies have shown that depending on the used plant material, the most suitable extraction solvent that can be used to determine polyphenol content and antioxidant capacity is acetone 50% for *Salacia chinensis* L. root (Ngo et al. 2017), acetone 60% for brewers' grains from *Hordeum vulgare* L. seeds (Meneses et al. 2013), acetone 100% for *Vaccinium myrtillus* L. leaf (Ceylan et al. 2017), ethanol 60% for *Cinnamomum cassia* (L.) J.Presl bark (Dvorackova et al. 2015), ethanol 100% for *Davidsonia pruriens* F.Muell fruit (Chuen et al. 2016), methanol 50% for *Allium sativum* L. husk (Kallel et al. 2014) and for *Ficus carica* L. seeds (Nakilcioğlu-Taş and Ötleş, 2021) and methanol 90% for *Helianthus annuus* L. florets (Ye et al. 2015).

While some studies have focused on optimizing the extraction of polyphenols from *Certanoia siliqua* L. seeds, none have focused on the leaves. There is therefore still no information on the effect of solvents of different polarities on the extraction of carob tree leaf polyphenols and their antioxidant activities. Consequently, this study aimed to determine, as a function of the extraction solvent (100% methanol, 100% ethanol, 100% acetone, 50% (v/v) aqueous methanol, 50% (v/v) aqueous ethanol and 50% (v/v) aqueous acetone), total polyphenol and flavonoid contents of carob tree leaves, and their relationships with their antioxidant activity (DPPH radical scavenging activity).

2. Material and Methods

2.1. Sample preparation

Carob tree leaves were harvested during the flowering period in October 2022 in Tlemcen (Algeria), geographic coordinates 35°04'06.6 "N 1°25'48.6 "W. They were thoroughly washed and then sent to the Pharmacognosy Laboratory of the Faculty of Medicine at Tlemcen for identification, leaf removal and fragmentation. They were then dried at room temperature for ten days, protected from light and humidity. After that, leaf fragments were ground using a mortar, in preparation for analysis.

2.2. Extraction process

Solid-liquid extraction process were used for the extraction of antioxidant compounds from carob tree leaves according to the protocol of Nakilcioğlu-Taş & Ötleş (2021) with slight modifications. Six different solvents were used: 100% methanol, 100% ethanol, 100% acetone, 50% (v/v) aqueous methanol, 50% (v/v) aqueous ethanol and 50% (v/v) aqueous acetone. 3 g of sample was mixed with 15 ml of solvent. The mixture was then stirred at 50°C for 90 minutes in a water bath (Memmert WNB 7, Germany) before filtering through filter paper. The volume of the extract aliquot was made up to 15 ml. Liquid extracts were stored at 4°C until analysis.

2.3. Determination of total polyphenols and total flavonoïds

Total polyphenols (TPs) and total flavonoids (TFs) determoination was carried out by spectrophotometric method in accordance with the protocol described and validated by Matić et al (2017). For total polyphenols, the standard used was gallic acid diluted in methanol (1, 10, 50, 100, 200, and 500 mg/L). 20 µL of standard or of the polyphenol sample, 1580 µL of distilled water, 100 µL of Folin-Ciocalteu reagent and 300 µL of Na_2CO_3 (200 g/L) were added to a glass tube. These solutions were vortexed (VWR® VV3, Germany) and incubated at 40°C for 30 min in a water bath. Absorbance was measured at 765 nm against a blank (containing 20 µL of distilled water instead of the prepared solution) using a UV-Vis spectrophotometer (Optizen 2120 UV, Korea). Results were expressed as milligram gallic acid equivalents per gram of dry matter (mg GAE /g DM). For total flavonoids, the standard used was catechin diluted in methanol (1, 10, 50, 100, 200, and 500 mg/L). 800 µL of distilled water, 200 µL of standard or sample and 60 µL of $NaNO_2$ (5%) were introduced into a glass tube. 60 µL of $AlCl_3$ (10%) were added after 5 min, then 400 µL of NaOH (1 mol/L) and 480 µL of distilled water were added after 6 min. The solution was stirred with the Vortex. Absorbance measurements were performed using spectrophotometer at 510 nm against a blank (containing 200 µL of distilled water instead of the prepared solution). Results were expressed as milligram catechin equivalents per gram of dry matter (mg CE/g DM).

2.4. Determination of antioxidant activity: Free radical scavenging method

The effect of antioxidant compound on radicals was assessed by DPPH according to the procedure described by Brand-Williams et al (1995), Sánchez-Moreno et al (1998), Kim et al (2015) and Selka et al. (2022). 0.1 ml standard ascorbic acid or sample diluted in methanol (1.8, 2.9, 4.9, 7.8, 12.7, 17.3 mg/L) was added to 3.9 ml DPPH freshly prepared in methanol (25 mg/L). Absorbances at 515 nm were measured after 30 minutes in the dark (until the reaction reached plateau). The Radical Scavenging Activity (RSA) was calculated as a percentage using the following equation:

$$\text{DPPH RSA (\%)} = [1 - (A_{sample}/A_{control})] \times 100$$

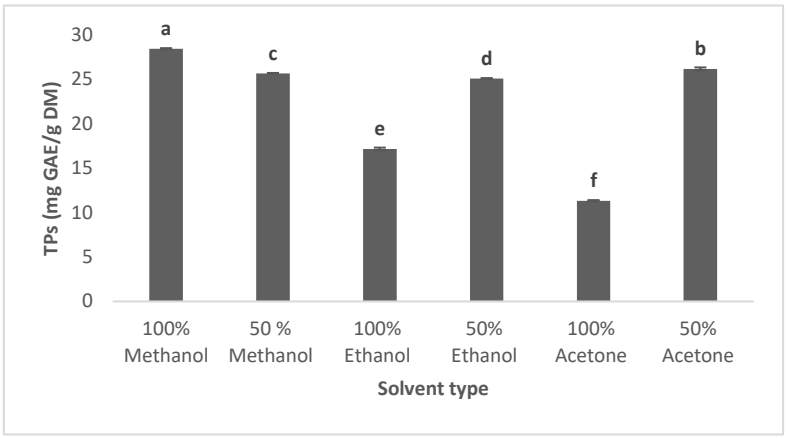
2.5. Analytical statistics

Results were expressed as mean value ± standard deviation, as each measurement was performed three times. To determine differences between values, ANOVA and Tukey test were used on IBM SPSS Statistics Trial Version 29.0.1.0 at a significance level of $p<0.05$. To determine the correlation between variables, Pearson correlation test was also used.

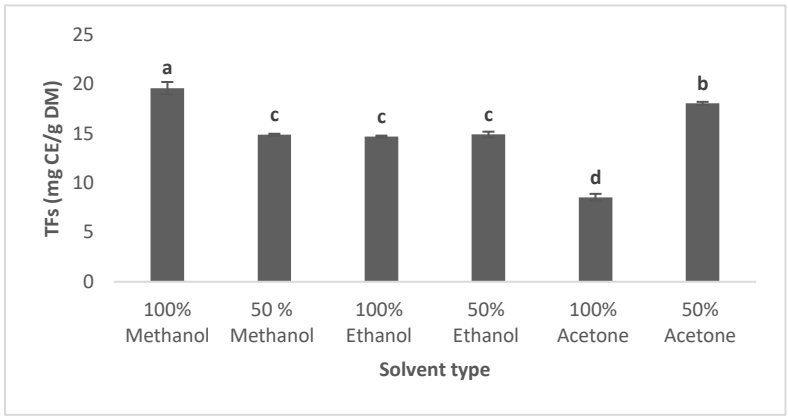
3. Results and Discussion

3.1. Total polyphenol, total flavonoid and antioxidant activity of the carob tree leaf extracts

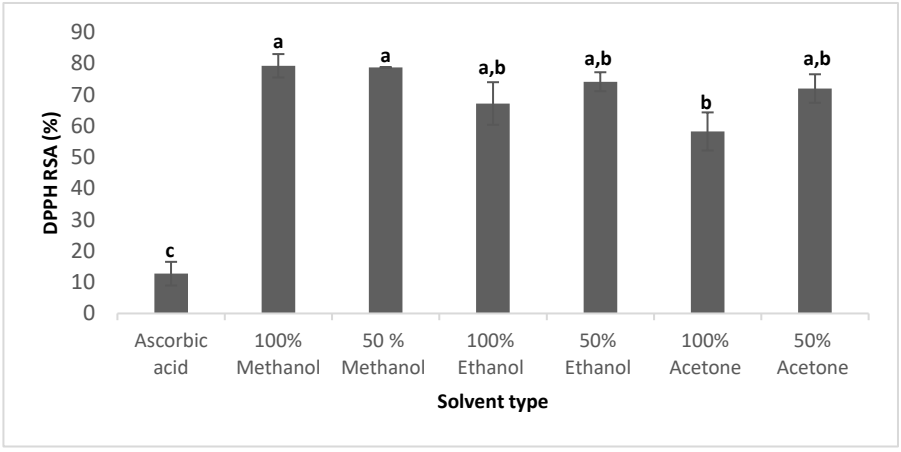
In this study, solvents of different polarities were used to extract polyphenols and antioxidant compounds from carob leaves. As shown in Figure 1 A, B, total polyphenol and flavonoid contents varied between extracts ($p<0.05$). For TPs, the linear trend curve for gallic acid followed this formula $y = 0.001 x - 0.0002$ with a correlation coefficient $R^2 = 0.999$, the deduced contents vary in the following ascending order: 100% acetone < 100% ethanol < 50% ethanol < 50% methanol < 50% acetone < 100% methanol ($p<0.05$). Whereas for TFs, the linear catechin trend curve followed the following formula $y = 0.0008 x + 0.0175$ with $R^2 = 0.991$ and contents varied in the following ascending order: 100% acetone < 100% ethanol =50%ethanol=50% methanol



A



B



C

Figure 1. TPs **A**, TFs **B** and DPPH radical scavenging activity (% inhibition) **C** values of carob tree leaves extracted with various solvents (n = 3 ± S.D.). Values marked with the different lowercase letters (a–f) are significantly different from each other at $p<0.05$.

< 50% acetone < 100% methanol ($p<0.05$). It can be said that the variations in TPs and TFs content between extracts showed the same general trend. Being the least polar solvent, 100% acetone was the least efficient in extracting this group of molecules, these same results were found in Nakilcioğlu-Taş & Ötleş's (2021) study on the influence of solvents on the TPs and TFs of fig kernels. But by adding distilled water, its extraction efficiency increased considerably becoming better than that of

wetted alcohols, indeed 50% acetone was the solvent that extracted the most TPs and TFs from bunga kantan in the study of Wijekoon et al. (2011) and from various vegetables in the study of Xu & Chang (2007), and 60% acetone for brewer's spent grains in the study of Meneses et al. (2013). For carob leaves, it was the 100% methanol that allowed to have the optimal content of PTs and FTs, these results approaching those reported in the study by Çelebi Sezer et al. (2017) on dried mushrooms where the best solvent was 80% methanol and those reported in the study by Ye et al. (2015) on sunflower florets where the best solvent was 90% methanol. The chemical composition of these plant parts made this solvent the preferred choice.

In this study, carob tree leaves were obtained from north-west Algeria, their TPs ranged from 11.33± 0.08 mg GAE/g DM in 100% acetone to 28.43± 0.08 mg GAE/g DM in 100% methanol, and their TFs ranged from 8.55± 0.34 mg CE/g DM in 100% acetone to 19.59± 0.63 mg CE/g DM in 100% methanol. The study of Elbouzidi et al (2023) revealed that TPs of carob tree leaves ethanolic obtained from northeastern Morocco were 96.98 ± 1.15 mg GAE/100 g DW and TFs were 5.92 ± 0.06 mg RE/100 g DW. Environmental factors, including harvesting season, extraction method and storage conditions, had a significant impact on phenolic composition.

All studied solvents had significantly higher antioxidant activity than ascorbic acid ($p<0.05$). The results of the DPPH test followed the same trend as for polyphenols and flavonoids, except for 50% methanol, which with 100% methanol gave the highest inhibition percentages :78.74± 2.99% and 79.29 ± 3.75% respectively ($p<0.05$) (Figure 1C). Methanol at 50% enabled the extraction of compounds with strong antioxidant activity, since the latter depending not only on the concentration of polyphenols, but also on their structure and their nature in the extract. On the other hand, this solvent was found to have the strongest antioxidant activity in the study of Kallel et al. (2014) on garlic husk and in that of Nakilcioğlu-Taş and Ötleş (2021) on fig kernels. Like TPs and TFs, antioxidant activity was stronger with 100% methanol, as reported in various studies on the same plant part (Mimoun et al. 2022, Madi et al. 2023). Carob tree leaves extracted with 100% acetone showed the lowest value (58.28%) which was positively related to TPs and TFs, this was also reported in the literature (Ngo et al. 2017, Nakilcioğlu-Taş & Ötleş 2021). Finally, there was no statistical difference with 50% acetone, 100% ethanol and 50% ethanol extracts in terms of the antioxidant capacities ($p<0.05$), which moreover, they remained close to those of methanolic extracts by their close polarities.

3.2. Correlation analysis between polyphenols and antioxidant activity

Correlation analyses were carried out between polyphenols contents and the antioxidant activity (Table I). Significant linear correlations were observed between TPs, TFs and DPPH values ($p<0.05$). Although flavonoids were a sub-group of polyphenols, the correlation between TPs and TFs ($r = 0.537$) was significant only at $p<0.05$, providing evidence of the presence of polyphenols other than flavonoids. TPs values were strongly positively correlated with DPPH values ($r = 0.892$) ($p<0.01$). This showed that polyphenols contributed to antioxidant capacities of extract samples. This indicates that most polyphenols in carob tree leaves were capable of reducing H+ ions. Statistically significant correlations were determined between TFs and DPPH values ($r=0.623$) ($p<0.01$). This proved that flavonoids significantly affected antioxidant capacities of the extracts. Several studies in the literature had also clearly established a close relationship between polyphenol content and antioxidant capacity (Xu &Chang 2007, Ghasemzadeh et al. 2015, Ye et al. 2015).

Table I. Correlation between TPs, TFs and antioxidant activity of carob tree leaf extracts.

Pearson's correlation (N=18)	TPs	TFs	DPPH RSA (%)
TPs	1		
TFs	0,537*	1	
DPPH RSA (%)	0,892**	0,623**	1

* The correlation was significant at 0,05
** The correlation was significant at 0,01

4. Conclusion

The results obtained in this study showed that carob tree leaves were a natural source of bioactive compounds. It was also found that extraction with solvents of different polarities affects the TPs, TFs and antioxidant capacity of carob tree leaf extract. Polyphenols and antioxidant compounds extractability from carob tree leaves was optimized by pure methanol. Furthermore, significant positive correlations were determined between TPs, TFs and antioxidant capacities of these extracts. Carob tree leaves could be considered as a source of important phytochemicals with antioxidant properties that could have beneficial effects on health. These results also indicated that carob tree leaf extract obtained using an appropriate extraction solvent could enhance the protective effect against oxidative damages associated with free radicals.

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Author Contribution

Concept, design, resources, analysis, interpretation and editing were carried out by Siham BABA AHMED. Monitoring and critical reviews were led by Mohammed Adil SELKA. Supervision was provided by Ilham LAHFA.

Conflicts of Interest

All authors declare no conflict of interest.

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Imperative Role of Natural Product Chemistry in Cosmeceutical R&D – Phytonanocosmeceuticals*

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bstract

Natural product chemistry has always been attractive for drug and cosmetic industries as natural products can address these industries very well. By consumer demand, the cosmetic industry is looking for innovative, safer, more effective, and environmentally friendly products. In this sense, a relatively new concept of cosmetics has emerged under "cosmeceuticals or dermocosmetics/medcosmetics". Cosmeceuticals are briefly defined as a subclass of cosmetics that contain drug active substances or bioactive natural products with enhanced efficacy for therapeutic or cosmetic purposes. They are also described as a combination of cosmetics and pharmaceutics or medical-grade cosmetics, which particularly enhance skin penetration and the restorative effect of active ingredients in cosmetic formulations. On the other hand, nanotechnology has become another exciting area in cosmetics as nanoformulations enhance skin penetration. Therefore, we have been working on research and development of novel phyto-based cosmeceuticals *via* extensive screening studies on plant extracts and purely natural substances using in vitro (enzyme inhibition, etc.), *in silico* (molecular docking and toxicity screening), and cell-based assays. In this regard, an anti-acne formulation based on a number of plant extracts tested against *Propionibacterium acnes* has been developed by our group. Besides, an antimicrobial formulation as an oral spray for mouth defense is currently a commercial product. We have also been studying nanofiber formulations loaded with plant extract with wound healing effects. All our ongoing studies on discovering novel active natural ingredients for cosmeceutical purposes have so far yielded three patents and four patent applications and commercialized a final product. In the present mini review, examples of phyto-based nanocosmeceuticals and nanoingredients will be underlined.

Key Words: Natural product chemistry, phytocompound, nanocosmetic, cosmeceuticals

1. Introduction

Phytocosmetics, also known as plant-based cosmetics, represent a fascinating and rapidly growing beauty and skincare industry segment. In today's world, where consumers increasingly seek natural, eco-friendly, and sustainable alternatives, phytocosmetics have emerged as a compelling choice. These products harness the power of botanical extracts, plant-based ingredients, and natural compounds to promote healthy and radiant skin, hair, and overall well-being. With a deep-rooted

connection to traditional herbal remedies and a focus on the inherent benefits of plants, phytocosmetics offer a holistic approach to beauty that emphasizes the harmony between nature and human skin care (Ahmed et al., 2022). Botanical ingredients in cosmetics are natural, plant-based substances commonly used in skincare, haircare, and beauty products (Mohammad et al., 2018). These ingredients have gained popularity in the cosmetic industry due to their perceived safety, efficacy, and the growing consumer demand for natural and eco-friendly beauty solutions (Kanlayavattanakul and Lourith, 2018). In the current mini review, we will briefly mention the relationship between botanical compounds and nanomaterials in cosmetic formulations.

2. Botanical ingredients in cosmetics

Botanical ingredients, known for their potential to benefit the skin, encompass a wide variety of plant extracts, oils, and powders derived from sources like herbs, fruits, flowers, seeds, and roots. Examples include aloe, lavender oil, chamomile extract, green tea extract, and many more. They can offer antioxidant protection, anti-inflammatory properties, hydration, skin-soothing, and anti-aging effects, etc. (Antignac et al., 2011; Ferreira et al., 2021). Various plants have unique properties that cater to specific skincare needs (Lianza et al., 2020; Goyal et al., 2022). Some botanicals, like *Rosa canina* L. (rosehip) and *Melaleuca alternifolia* (Maiden & Betche) Cheel. (tea tree) oils, are recognized for their anti-aging and acne-fighting properties (Segueni et al., 2022). They can also help reduce the appearance of wrinkles and blemishes.

Botanical ingredients are generally considered safer and have fewer side effects than synthetic chemicals, making them popular for individuals with sensitive or allergy-prone skin. Some plant extracts and natural molecules possess natural preservative properties, which can help extend the shelf life of cosmetics without the need for synthetic preservatives (Papageorgiou et al., 2010; Novak et al., 2021; Rybczyńska-Tkaczyk et al., 2023). Sourcing botanical ingredients can be more sustainable and environmentally friendly than specific synthetic alternatives, aligning with the demand for eco-conscious beauty products (Serra et al., 2023). The consumer preference for natural and plant-based products has driven the cosmetics industry to include botanical ingredients in a wide range of products, from cleansers and moisturizers to shampoos and perfumes (Bauman et al., 2007). The efficacy and safety of botanical ingredients are often backed by scientific research and clinical studies (Avonto et al., 2018). Researchers investigate the bioactive compounds within these plants and their effects on the skin. The use of botanical ingredients in cosmetics is subject to regulatory oversight in various countries. Manufacturers must ensure that their products meet safety and labeling requirements.

3. Nanomaterial-based cosmetics

Nanotechnology is a multidisciplinary field of science, engineering, and technology that deals with nanoscale materials, devices, and systems, typically at the level of individual atoms and molecules. The prefix "nano" refers to one billionth of a meter or 10-9 meters, and nanotechnology involves working with structures and components that are typically between 1 and 100 nanometers in size. At the nanoscale, materials often exhibit unique and enhanced properties compared to their bulk counterparts. This can include changes in mechanical, electrical, thermal, optical, and chemical properties. Nanotechnology has revolutionized the field of medicine as well as cosmetics (Gupta et al., 2022; Basudkar et al., 2022). Nanotechnological drug dosage forms, often called nanomedicine or nanopharmaceuticals, represent a groundbreaking drug delivery and therapy

approach (Patra et al., 2018), which can be applied to cosmetic formulations. These innovative formulations utilize nanotechnology to design and manipulate drug/cosmetic-active carriers and delivery systems, enabling precise targeting and controlled release of therapeutic and dermocosmetic agents. Nanocosmetics, often referred to as nanotechnology-based cosmetics, represent an innovative and rapidly evolving sector of the beauty and skincare industry (Shokri, 2017; Vaishampayan and Rane, 2022). These products leverage nanotechnology, which involves working with materials at the nanoscale, to create novel and highly effective skin care solutions (Santos et al., 2019; Dubey et al., 2022). Nanocosmetics offer several unique features and advantages, including:

Enhanced penetration: Nanoparticles can penetrate the skin more effectively, allowing active ingredients to reach deeper layers (Bucci et al., 2018). This enhances the effectiveness of skin care products.

Improved stability: Nanotechnology can enhance the stability of certain ingredients, extending the shelf life of products and ensuring they remain effective over time (Zhang et al., 2023).

Targeted delivery: Nanoparticles can be designed to release active ingredients at specific times or locations, allowing for precise and targeted treatment of skin issues (Di Stefano, 2023).

Increased efficacy: The reduced particle size allows for a more even distribution of active ingredients on the skin, resulting in better overall efficacy (Patil et al., 2015; Rama & Ribeiro, 2023).

Cosmetic and aesthetic benefits: Nanocosmetics can provide aesthetic benefits such as smoother textures, better coverage, and improved color dispersion in makeup products (Zouboulis et al., 2019).

Reduced irritation: Nanoparticles can reduce the potential for skin irritation, making these products suitable for individuals with sensitive skin (Bai et al., 2023).

Nanocosmetics encompass a variety of product types that incorporate nanotechnology for enhanced performance, improved texture, and targeted effects (Raszewska-Famielec and Flieger, 2022). Some common types of nanocosmetic products are as follows:

Nanoparticle sunscreens: These sunscreens contain nanoparticles, such as zinc oxide or titanium dioxide, which provide effective UV protection without leaving a visible white residue on the skin. The small particle size allows for a cosmetically elegant appearance (Dréno et al., 2019; Lin et al., 2024).

Nanomaterial-based make-up: Nanotechnology is used to create makeup products like foundations, powders, and eyeshadows. Nanoparticles improve color dispersion and create a smoother, more even texture (Aziz et al., 2019; Santos et al., 2019).

Antiaging serum and creams: Anti-aging skincare products often incorporate nanoparticles to enhance the delivery of active ingredients like peptides, antioxidants, and retinoids (Bellu et al., 2021; Jin et al., 2023). This improves their penetration into the skin and effectiveness in reducing signs of aging.

Nanosome and liposome-based skin care: Nanosomes and liposomes are nanoscale carriers encapsulating active ingredients (Fakhravar et al., 2016; Cheng et al., 2020). They are used in a range of skincare products, such as moisturizers, serums, and creams, to improve ingredient delivery and stability.

Nanoemulsions: Nanoemulsions are used to create lightweight and easily absorbed cosmetic formulations (Ngan et al., 2015). They are commonly found in products like moisturizers, cleansers, and serums.

Nanoparticle-enhanced haircare: Nanotechnology is employed in hair care products to improve the delivery of vitamins, conditioners, and other beneficial ingredients to the hair and scalp. This can lead to healthier, more lustrous hair (Heng et al., 2011).

Nanogels and nanocapsules: These nanocarriers are used to encapsulate and deliver active ingredients in a controlled manner (Marchiori et al., 2017; Cardoso et al., 2019). They are commonly found in products designed for targeted skincare and treatments.

Nanoparticle-infused nail products: Some nail polishes and treatments incorporate nanoparticles for improved durability, shine, and wear resistance (Trombino et al., 2016; Flores et al., 2017).

Nanoparticle-containing fragrances: Nanotechnology is beneficial in creating fragrance formulations that provide longer-lasting scent, better adherence to the skin, and improved fragrance stability (Capasso Palmiero et al., 2020; Hu et al., 2021; Wang et al., 2022).

It is important to note that using nanotechnology in cosmetics has raised questions and concerns regarding safety and regulatory oversight. Some people worry about potential health risks associated with using nanoparticles in cosmetics, and regulatory agencies in various countries have implemented guidelines and regulations to ensure the safety of nanocosmetic products.

4. Conclusion

Nanotechnological dosage forms have significantly advanced the cosmetic industry, leading to the development of products that offer enhanced benefits and a more enjoyable user experience. As technology progresses, cosmetic companies need to balance innovation with rigorous safety testing to ensure the well-being of consumers. On the other hand, using nanoparticles in various products has raised concerns about potential health and environmental risks. Ensuring the safety of nanotechnology is an ongoing challenge, and regulatory agencies are working to establish guidelines for the responsible use of nanomaterials. Consumers interested in nanocosmetics should be informed about the ingredients and technologies used in these products and any manufacturer safety assessments. As with any cosmetic purchase, individual skin type and potential sensitivities should be taken into account, when selecting and using nanocosmetic products.

Nanotechnology holds great promise for addressing complex challenges in various fields, such as creating more powerful and energy-efficient electronics, developing targeted drug therapies, and improving the efficiency of cosmetics. Nanotechnology continues to be a rapidly evolving field with far-reaching implications for science, industry, and society. Researchers, policymakers, and the public are all engaged in ongoing discussions about its potential and the responsible development of nanotechnology.

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Conflicts of Interest

The authors declare no conflicts of interest.

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