

GC-MS ANALYSIS FOR CHEMOTAXONOMIC CHARACTERIZATION
AND BIOLOGICAL ANALYSIS OF *ARTEMISIA ANNUA L.*

Sana Begum

Masters in Organic Chemistry University of Karachi

sana_iqbal11290@hotmail.comDOI: <https://doi.org/10.5281/zenodo.21902585>**Keywords**

Artemisia annua L.; essential oil; GC-MS; FTIR; chemotaxonomy; phytochemical analysis; antimicrobial activity; hydro-distillation; β -caryophyllene; artemisia ketone

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Corresponding Author: *

Sana Begum

Abstract

Artemisia annua L. is an important medicinal plant widely recognized for its diverse phytochemical constituents and broad range of biological activities. The chemical composition, functional-group characteristics and antimicrobial activity of the essential oil of *A. annua* harvested from Mansehra, Pakistan were studied. Hydro-distillation method was used for the extraction of essential oil from the plant material with the help of Clevenger apparatus. The qualitative and quantitative characterization of the volatile compounds was performed by gas chromatography/mass spectrometry (GC-MS) and major functional groups in the oil were identified by the Fourier transform infrared (FTIR) spectroscopy. The analysis by gas chromatography-mass spectrometry (GC-MS) revealed the presence of 28 constituents accounting for about 95.5% of the total detected constituents. Chemically the essential oil was found to be very diversified and got higher percentage of the constituents (26.14% artemisia ketone, 24.70% β -caryophyllene, 10.66% camphor and 8.05% eucalyptol). FTIR confirmed the presence of hydroxyl, aliphatic C-H and carbonyl and C-O functional groups by looking at their characteristic absorbance bands. The biological activity essential oil tested by agar well diffusion with minimum inhibitory concentration (MIC) against selected microorganism. The essential oil was inhibitory for the tested microorganisms *S. typhi*, *P. aeruginosa*, *E. coli*, *S. aureus*, *B. subtilis*, *P. mirabilis* and *Candida albicans*. The overall conclusion is that *A. annua* EO has a wide range of volatile chemical constituents and has some antimicrobial activity. The GC-MS coupled with FTIR system offers valuable chemical data of the essential oil of *A. annua* and it facilitates the potential use of the essential oil in chemotaxonomic and biological research.

1. INTRODUCTION

Natural products have historically played an important role in the discovery and development of medicinal agents. The bioactive substances derived from plant, microbes, marine and other biological sources can be a rich source of compounds with pharmaceutical and biological applications, which are of diverse chemical

nature. Harvey (2008) reported that the biological activity and structural diversity of natural products is particularly important for drug discovery. Secondary metabolites typically play a role in the interactions with the environment and in defense mechanisms, while primary metabolites are essential for the main physiological processes (growth, development and

reproduction). These metabolites are distinguished metabolites, which gives an organism a chemical tool to deal with environmental and biological stresses, according to Katz and Baltz (2016). Natural products are thus structurally heterogeneous and are useful for phytochemical, pharmacological and biological studies.

The current significance of natural products in the discovery of new drugs is reflected in the number of therapeutic agents derived directly or indirectly from natural products. The natural products and their derivatives have made significant contributions to the discovery of clinically important drugs in the past few decades by way of research, as reported by Newman and Cragg (2020). The importance of plant derived secondary metabolites lies in the fact that plants produce many structurally diverse metabolites that are synthesized as secondary metabolites and include terpenoids, flavonoids, alkaloids, phenols, coumarins and other specialized metabolites. These compounds are known to have antimicrobial, antioxidant, antiparasitic, antiviral, anti-inflammatory and other biological activities. Therefore, systematic study of medicinal plants is of paramount significance to detect its bio-components and to explore the medicinal and phytochemical applications of the medicinal plants.

The volatile constituents and the essential oils are one of the most widespread and interesting groups of plant secondary metabolites due to their complexity and biological effects. The essential oils of aromatic plants are usually composed of a complex mixture of volatile compounds and are typically high in the proportion of monoterpenes, sesquiterpenes, oxygenated terpenoids, and, in a few species, phenylpropanoids. Species can be composed of non-constant composition. Alternatively, it could differ based on genetic factors, climatic region, environmental conditions, developmental stage, plant part, and extraction method. Variations are of special significance when essential oils are examined for biological activity as changes in the proportions of individual constituent(s) can mean variations in overall biological action.

Thus, chemical data for essential oils can be useful in the pharmacological study of plants as well as in plant taxonomy. Chemotaxonomy is the utilization of the chemical characteristics (secondary metabolites in particular) for the identification and differentiation of plant taxa. Chemical data can be very helpful in addition to morphology to characterize populations or nearby taxa where the variation among them cannot be explained by the morphology. In aromatic and medicinal plants, the occurrence of characteristic terpenoids and other secondary metabolites may be informative as they can be species- or population-associated, allowing their distribution to be used for investigating the plants. Therefore, the constituents of essential oils can be characterized and aid with the development of chemical fingerprints which might be of chemotaxonomic value.

One of the most valuable techniques to study the volatiles chemistry of medicinal plants is gas chromatography-mass spectrometry (GC-MS). The gas chromatography alone is able to separate individual volatile constituents based on some of their physicochemical characteristics, while the mass spectrometry alone is able to give spectral information to help in identification. The combination of these two techniques can give the complex essential-oil mixtures a rather detailed characterization. Mass spectra in combination with reference mass spectra or published mass spectra and retention indices can aid identification of individual components. In the scope of chemotaxonomic studies the media profile emerges as the profile typical of a plant sample and can be compared with the profiles of other populations or geographical origins.

Artemisia is an important genus of plants of medicinal and aromatic importance in Asteraceae. For several reasons, the species of this genus have been traditionally applied in various medicinal systems and are known for possessing a large number of phytochemical properties. There are some species of particular interest to study due to their unique secondary metabolites and medicinal properties, such as *Artemisia annua* L. (sweet wormwood or annual wormwood). Notably this species has captured special interest

due to its role as the natural origin of the sesquiterpene lactone, artemisinin with key anti-malarial properties.

Bhakuni et al. (2001) reported on the secondary metabolites of *A. annua* and pointed out the significant chemical diversity in the species. Besides artemisinin, several other secondary metabolites are present in *A. annua*, such as flavonoids, terpenoids, coumarins and volatile components. They are chemically varied compounds, and the presence of those types of compounds are the basis for studying both the phytochemical and pharmacological characteristics of a plant. The chemical components of *A. annua* are of great interest since biochemical activity may be due to other active ingredients as well as artemisinin.

Other compounds reported from *A. annua* are flavonoids, an important group of constituents. Ferreira et al. (2010) did a review of the flavonoids of *A. annua* and explained the structural variety of the flavonoids present in the species. The constituents have garnered interest for their possible biological properties including antioxidants. Since flavonoids are co-occurring with other secondary metabolites such as terpenoids in *A. annua*, their biological properties might be induced by the interaction of more than one type of phytochemical. It is an important point to note that this chemical complexity comes into consideration as a factor to consider in interpreting the biological assays done by using extracts or essential oils of plants.

The phytochemical diversity of *A. annua* is paralleled by wide-ranging biological activities reported. Publishing in herbal medicine, Lubbe et al. (2012) reviewed the traditional use, phytochemistry and pharmacology of *A. annua* and highlighted the fact that it is rich in biologically important constituents and has a long history of medicinal use. The plant has been used traditionally for fever and several other ailments and being the subject of research, explored for its antimicrobial, antiparasitic, antioxidant, anti-inflammatory and other activities. This, together with the traditional and

experimental evidence, has maintained the scientific interest in the species.

A. annua is mostly known for artemisinin and its derivatives for their antimalarial properties. Investigation and development of artemisinin-based medicines made *A. annua* one of the most impactful medicinal plants in recent years in malarial drug development. The potential of the species, however, is not limited to the treatment of malaria. Pittiticide, the activity of artemisinin and compounded against protozoan infection, which is not limited to malaria, was discussed by Efferth and Oesch (2017) and uncovered that metabolites of *A. annua* still continue to elicit interest in terms of their antimicrobial activity, which could also target parasites. Derda et al. 2016 have also studied *A. annua* preparations with regard to *Acanthamoeba* infection and they mentioned the biological activity of preparations made with plants in experimental models.

A. annua was also studied for its antioxidant and anti-inflammatory properties along with its antiparasitic effects. Ferreira et al. (2010) demonstrated flavonoids in *A. annua* are of interest since these have antioxidant activities. The diversity of secondary metabolites correlated to various activities have also been reported by Bhakuni et al. (2001). Overall, these observations indicate that the medicinal characteristics of *A. annua* are more likely to be the result of a wide range of phytochemicals rather than solely artemisinin.

Anhydride nature of *A. annua* has been also reported for its antimicrobial activity. Al-Ani et al. (2014) showed that the biological activities of the *A. annua* preparations made from different sources differed and that activity could vary depending on the nature and origin of the plant materials and the method of extraction. This variation is significant as the biological activity of the plant preparation is highly dependent on the chemical composition. Therefore, the different antimicrobial activity of the preparations from *A. annua* may be due to variations in the leaching of volatile terpenoids and other secondary metabolites.

A. annua is of special interest for antimicrobial studies due to the presence of volatile components which can react with microbial cells in the essential oil extract. They studied the chemical composition and antimicrobial activity of the essential oil of *A. annua* (Radulovic et al., 2006), and showed that the essential oil of *A. annua* is a chemically complex mixture of volatile constituents with antimicrobial activity. According to the authors' results, it is important to study the constituents of an essential oil when studying the biological effects.

Rosa et al. (2015) have studied the antibacterial property of *A. annua* oil and its active ingredients against some selected food borne pathogens. Their study showed inhibitory activity of the essential oil and pointed to the potential role of its key components (camphor and artemisia ketone) in the antibacterial activity. The constituents involved in essential oils were suggested as interacting with the microbial membranes which may decrease or increase the permeability of the membrane, resulting in a negative or positive impact on cellular integrity and microbe survival. The findings give a scientific foundation for the investigation of the essential oil of *A. annua* and its chemical composition along with its anti-microbial properties.

The chemical composition of essential oil obtained from *A. annua* can also significantly vary from one sample to another. This variation could be correlated with genetic variation, geographical origin, environmental conditions or other factors that may affect the secondary metabolism. So, identification and quantification of the volatile constituents are of importance for determining chemical profile of a specific population of an individual plant. In this case, the use of GC-MS is seen as a good tool for the characterization of major and minor components of the essential oil. The obtained profile can then be compared with previously published chemical profiles to estimate similarities and differences that could be of chemotaxis interest.

The relevance of the present study is in the fact that the chemical profile of a medicinal plant can

provide a qualitative connection between herb identification as a taxonomic unit and phytochemistry, biological properties. Volatile biomarker patterns can be used as chemical fingerprints and the biological evaluation can provide a hint that the detected chemical constituents are linked to antimicrobial activity. These can be combined to provide a more complete assessment than chemical characterization or biological testing.

The aim of the present investigation was to understand the volatile components of *A. annua* essential oil and also evaluate its chemotaxonomic value by analyzing the oil using the GC-MS analysis. The main and minor components of the essential oil and the relative amounts of the most abundant were identified. The results of the chemical analysis were also complemented by Fourier-transform infrared (FTIR) spectroscopy data to give more information on the functional groups found in the analyzed oil. According to the thesis, 28 constituents of the essential-oil composition were identified by gas chromatography - mass spectrometry (GC-MS) accounting for around 95.5% of the identified components. The main constituents that were reported were: artemisia ketone, 26.14%; β -caryophyllene, 24.70%.

The biological component involved the evaluation of antimicrobial activity (against selected bacterial microorganisms and fungal microorganisms) of the essential oil extracted. The thesis shows that it has inhibitory activity against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus mirabilis* and *Candida albicans* and in the conditions used in the study it did not show inhibitory activity on *Streptococcus mutans*. By analysing the biological endotoxemia observed in conjunction with the chemical characterisation using GC-MS, the chemical structure of the oil can be taken into account.

Thus, the present study aimed to characterize the essential oil of *Artemisia annua* via GC-MS, analyze its components based on a chemotaxonomic point of view and assess its antimicrobial activity. This work will involve

chemical profiling coupled with biological evaluation with an aim to contribute to the understanding of the phytochemical character of *A. annua* and possible relationship between its volatiles and activity. The information provided might serve as a benchmark for the development of the potential of *A. annua* as an essential oil source of biologically active natural products and for comparison with other populations and geographical region chemical studies.

2. Literature Review

2.1 Medicinal Plants and Their Importance

The use of medicinal plants has long been an important part of traditional medicine systems around the world and has been utilized for thousands of years for therapeutic purposes. They are not only important historically, but also for their capacity to synthesize a variety of secondary metabolites with pharmacological and biological properties. Traditional and herbal Medicine continues to be important vehicle to healthcare in lots of parts in the world (Chan 2003). It is noted that communities with greater traditional health care dependency show higher levels of continued use of medicinal plants, and where traditional health care is tightly linked with daily life, this may be reason to wonder about the chances of successful education and extension campaigns regarding medicinal plants.

This has raised increased interest in medicinal plants that has led to scientific studies of them. Kotnis et al. (2004) reported that growing interest in natural products with applications to human health. Plants can be utilized directly as traditional treatments or as sources for the production of compounds which are used in the development of pharmaceuticals. There is immense diversity of medicinal plants in India whose use in traditional medicine is of significant importance along with their usefulness in pharmaceutical applications as brought out by Bhagwati (2003).

A series of complementary disciplines are used in the scientific investigation of medicinal plants, such as botany, ethnopharmacology, phytochemistry, pharmacology and toxicology. Harsha et al. (2002) also pointed out that the

documentation of traditional medicinal plants and the search of their therapeutic properties were of utmost importance. These explorations can give scientific facts to traditional employments and can additionally assist with the discovery of new bioactive substances.

The genus *Artemisia* has been extensively studied among the medicinally important plant genera due to its wide geographical distribution, traditional applications and abundant therapeutic metabolites. There are many aromatic species in the genus which have been extracted and investigated for antimicrobial, antioxidant, antiparasitic, anti-inflammatory activity and others.

2.2 The Genus *Artemisia*

The genus *Artemisia* is within the family Asteraceae (Compositae) and is one of the large group of aromatic plants in the family that is widely distributed throughout the world. Watson et al. (2002) studied the molecular phylogenetic of the subtribe Artemisiinae, which also covered the inclusion of the genus *Artemisia* and related genera. It is a genus with over 500 species, mainly occurring in the temperate parts of Asia, Europe and North America (Mehrdad et al., 2007).

Species of *Artemisia* are annual or perennial herbs, shrubs or small woody. There are a number of species with aromatic leaves and unique bitter or highly aromatic secondary metabolites. Some of the chemical diversity among the genus is the presence of terpenoids, flavonoids, coumarins, sesquiterpene lactones and other specialized metabolites (Abad et al., 2012).

Several traditional health care systems have been aware of the medicinal value of the genus. Various *Artemisia* species have been used for digestive diseases, for fever, in the treatment of infections, as an anti-inflammatory and for parasitic diseases. Bisht et al. (2021) pointed out that various biological activities of *Artemisia* species have been examined such as antimalarial, antimicrobial, antioxidant, antiparasitic, anticancer, antiviral and gastroprotective effects. Due to the large chemical diversity in this genus,

there is the potential to study plant-chemical relationships with regard to biological activity.

From a chemotaxonomic point of view, the genus is also important. There may be distinct volatile and non-volatile characteristics of different species of *Artemisia*. Chemical variation can be added to the species characterisation methods based on morphology and molecules. This feature of distinctive essential-oil constituents among the *Artemisia* species therefore provides a vital guide for chemical profiling and chemotaxonomic studies.

2.3 Botanical Characteristics of *Artemisia annua* L.

Artemisia annua L., or annual wormwood and qinghao, is an aromatic annual herb of the family Asteraceae. Widely used for its medicinal applications as a precursor of certain drug products, and widely known for its aromatic properties. Ferreira et al. 2005, defined *A. annua* as an annual herb that grows within its surroundings with considerable differences depending on its cultivar, environmental factors, and cropping conditions.

The plant tends to be erect with potential growth reaching about 30–100 cm under normal cultivation, but up to much higher lengths under optimum conditions (Ferreira et al., 2005). It has deeply divided or serrated leaves and profuse small yellow flowers in clusters. The study of the impact of agronomic management on *A. annua* yield and content of artemisinin by Laughlin (1993) provided a proof that cultivation conditions were a significant factor to plant characteristic and phytochemical accumulation.

Very tiny brown seeds are reproductive in structure. Bhakuni et al., (2001), mentioned that seeds are small and species is of high value as a medicinal plant. Botanical aspects of *A. annua* are significant for phytochemical research, because the secondary metabolites' concentration may differ in each plant organ, depending on its plant development, genotype and environmental conditions.

2.4 Origin and Geographical Distribution of *Artemisia annua*

The plant *Artemisia annua* is traditionally attributed to Asian zones specifically China, with extensive historical records of its medicinal application in these areas. Hsu (2006) traced back the history of qinghao's use in Chinese materia medica and revealed the historical significance of the plant in traditional Chinese medicine. Other countries where it is found are Vietnam, Myanmar, northern India and Japan (van Agtmael et al., 1999).

The geographic range of *A. annua* has spread from its native area. Wright and Linley (2010) outlined the use of *Artemisia* species in various regions and their therapeutic uses, and the use of the species has been reported from countries such as Vietnam, Romania, Tanzania, Australia, Turkey and Afghanistan in the literature of the thesis.

The geographical distribution should be taken into account especially when dealing with the phytochemistry of *A. annua*. Variations in environmental conditions, soil properties, temperature, vine age/height, farming practices and genetic diversity could affect secondary metabolite accumulation. The critical role genetic and cultivation factors play in determining the production of artemisinin was highlighted by Ferreira et al. 2005.

Geographical population differences are also important for essential-oil composition. The composition of essential oils of *Artemisia maritima* has been shown to be geographically variable (Jaitak et al. 2008). Similarly, Lin et al. (2004) reported differences in volatile-oil constituents of *Artemisia scoparia* from different growing areas. Chemical data on *Artemisia* species has been compared according to their geographical origin, which is concordant with the observations.

2.5 Nutritional Value of *Artemisia annua*

A. annua is mainly known for its medicinal importance but it was also investigated for its nutritional composition. The plant is a source of proteins, fats, vitamins and minerals (including

zinc and copper) reported by Ferreira and Janick (1996). The species are also chemically complex, with some biologically active secondary metabolites as well as nutritional components. Weathers et al. (2011) noted water- and alcohol-soluble material in *A. annua* and provided ash and moisture data for plant material. The ash level of the thesis is about 3.7 % and the moisture is 2.5 %. Nutritional composition is not a main focus of the present investigation but the results of this show the chemically varied aspect of the plant.

2.6 Phytochemistry of the Genus *Artemisia*

Artemisia is a chemically complex genus that has been confirmed by phytochemical studies. The bioactive essential oils of *Artemisia* were reviewed by Abad et al. (2012) and nine important classes of constituents were described, such as terpenoids, flavonoids, coumarins and acetylenic compounds. These compounds occur in different species in different abundance and distribution. Terpenoids, one of the most characteristic components of *Artemisia*. Monoterpenes, sesquiterpenes, oxygenated terpenoids and sesquiterpene lactones are generally present in aromatic species. These compounds are important because of their biological functions, as well as their chemical fingerprint and aromas for each species.

Like *Artemisia absinthium* which has been reported as containing thujone isomers and other volatiles. The traditional phytotherapeutic application of *A. absinthium* has been described by Guarrera (2005) and the chemical analysis of

the volatile compounds of *A. absinthium* (myrcene and trans-sabinyl acetate) by Daise et al. (2008).

Artemisia abrotanum is endowed with flavonoids, coumarins, as well as volatile compounds like 1,8 cineole, linalool, etc. The flavonoids of this species were investigated by Remberg et al. (2004) while anti-inflammatory plant activity was investigated with regard to medicinal plants such as *Artemisia* species by Tunon et al. (2006).

Liu et al. (2009) reported the presence of flavonoids, glaucolides, guaianolides and monoterpenoids in *Artemisia afra*. These constituents are responsible for the diversity of the species in terms of both its chemistry and biological importance.

The essential oil of *Artemisia maritima* contains volatile compounds such as 1,8-cineole, camphor and borneol which have been described in the literature (Jaitak et al., 2008). Importantly, the study also showed that there is variation in the essential-oil composition from different locations, which not only highlights the importance of chemical variation in chemotaxonomic studies but also reveals the relationship between the composition and color of the scents emitted.

In addition to the above, other constituents for *Artemisia scoparia* have been reported as α -pinene, 1,8-cineole, carvone, eugenol and some flavonoid compounds (Lin et al., 2004). The variation in chemical composition in the various species in the genus is indicative of the possibility of using secondary metabolites as chemical traits for comparative studies.

Table 1. Representative phytoconstituents reported from selected *Artemisia* species

Species	Representative constituents	Reference
<i>A. absinthium</i>	(Z)-Thujone, (E)-thujone, myrcene, trans-sabinyl acetate	Guarrera (2005); Daise et al. (2008)
<i>A. abrotanum</i>	Flavonols, coumarins, 1,8-cineole, linalool, thujyl alcohol	Remberg et al. (2004); Tunon et al. (2006)
<i>A. afra</i>	Flavonoids, glaucolides, guaianolides, monoterpenoids	Liu et al. (2009)
<i>A. annua</i>	Sesquiterpene lactones, flavonoids, artemisinin-related constituents	Han et al. (2007, 2008); He et al. (2009)

<i>A. maritima</i>	1,8-Cineole, camphor, borneol	Jaitak et al. (2008)
<i>A. scoparia</i>	α -Pinene, 1,8-cineole, carvone, eugenol, flavonoids	Lin et al. (2004)

The variety outlined above shows the need to have chemical profiling useful comparison of the various species of *Artemisia*. Other recent reviews also list terpenoids, flavonoids, coumarins and other groups as major ones accounting for the biological diversity of the genus.

2.7 Phytochemical Characteristics of *Artemisia annua*

The phytochemistry of *A. annua* has been extensively investigated because of the presence of artemisinin and numerous additional secondary metabolites. Bhakuni et al. (2001) have summarized and explained the biological significance of the secondary metabolites of *A. annua*. The species has sesquiterpene lactones, flavonoids, terpenoids, coumarins and volatile constituents.

Artemisinin is the best known secondary product of *A. annua*. Artemisinin biosynthesis and phytochemistry were reviewed by Brown (2010) and it was noted that artemisinin was mainly accumulated in the glandular trichomes of aerial tissues. The amount of Artemisinin is genotype dependent, varies by geographical location, weather and farming conditions.

According to Weathers et al. (2011), dried leaves of *artemisia* could contain varying level of artemisinin ranging from about 0.1% to 1.6%. This variation illustrates that phytochemicals can be affected by both genetic and environmental traits. When comparing geographical variation of plant material, such variation is important.

Yet another group of substances found in *A. annua* are flavonoids. The liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) method was used by Han et al. (2007) for identification of flavonoids in *A. annua*. Han et al. (2008) later used HPLC-DAD-ESI/MS to compare the flavonoid profiles. These trials illustrate the great variety of flavonoids that this plant contains.

He et al. (2009) focus on the chemical compositions and biological activities and reported compounds from *A. annua* for the various pharmacological potentials. The presence of artemisinin, flavonoids, terpenoids, and other components in *A. annua* would mean that the biological activity of *A. annua* preparations might result from multiple components in addition to artemisinin.

2.8 Essential Oil of *Artemisia annua*

The essential oil is one of the important volatile constituents of *A. annua*. The oil is complex mixture of monoterpenes, sesquiterpenes, oxygenated terpenoids and other volatiles. Abad et al. (2012) acknowledged the significance of essential oils of *Artemisia* genus and their relationship with various biological processes.

A. annua essential oil has variable chemical composition. Different compounds have been identified as the dominant chemical components in different investigations over geographical origin, plant material, cultivation methods, extraction methods etc. The essential oils of *A. annua* have been recently reviewed, and were reported to contain significant amounts of oxygenated monoterpenes and sesquiterpenes including artemisia ketone and camphor.

Konovalov and Khamilonov (2016) have made a review on the essential oil of *A. annua* and have highlighted active biological components present in the essential oil emphasizing the diversity of its chemical composition and the pharmacological potential.

The variability of essential-oil composition is of special significance with regard to chemotaxonomy. The geographical variation in the essential oil composition of *A. maritima* was established by Jaitak et al. (2008) and variation in the volatile constituents of *A. scoparia* was reported by Lin et al. (2004) from different growing areas. Chemical characterisation of the

genus is provided by these studies and offers examples of possible variability within the genus.

2.9 Chemotaxonomic Significance of Secondary Metabolites

Chemotaxonomy is the classification method supplemented with the use of chemical properties. For medicinal and aromatic plants, secondary metabolites can be of value in elucidating the relationships among species, populations or chemotypes. There is great variation in artemisia constituents such as terpenoids, flavonoids, coumarins and the like, so that a chemical comparison is appropriate.

Further genetic analyses of the subtribe Artemisiinae revealed the phylogenetic diversity inherent in *Artemisia* and related genera (Watson et al. 2002). Chemical characterization can give additional information to molecular and morphological characterization.

It is also advantageous to use essential oils for chemotaxonomic studies since they can be separated and identified as chemical profile. Combinations of monoterpenes, sesquiterpenes and their oxygenated counterparts can be used to provide chemical fingerprints, which may be characteristic of some groups of species or populations.

Jaitak et al. (2008) showed geographical differences in composition of essential-oils for *A. maritima*. Geographical variation of *A. scoparia* was also shown by Lin et al. (2004). The studies such as this show that the chemical composition may be affected by environmental and geographical factors and hence should be taken into account in understanding chemotaxonomy relationships.

A. annua is especially significant with respect to chemical characterization of its volatile compounds due to its high biological activity that derives from the high concentration of biological activity compounds in the species. Therefore the use of GC-MS based profiling can be used as a practical solution to identify the major and minor volatile constituents and then compare it with the reported chemical profile.

2.10 Pharmacological Activities of *Artemisia annua*

A. annua contains a large diversity of phytochemicals, which have been reported to have a variety of biological and pharmacological actions. They have antimalarial, anticancer, anti-parasitic, gastrointestinal, antimicrobial, anti-inflammatory, antioxidant and antiviral properties. The literature available suggests that several group kinds might add to these activities.

2.10.1 Antimalarial Activity

The antimalarial activity of *A. annua* is primarily associated with artemisinin. The finding of artemisinin was a break-through in malaria treatment especially for *Plasmodium falciparum* (Tu, 2015).

Van Agtmael et al., (1999) presented how a medicinal herb has been transformed into a registered antimalarial medicine. Artemisinin became increasingly important and led to the worldl widely cultivating *A. annua* for manufacturing pulp pharmaceutical drugs.

But studies have shown that the activity of the whole plant, which is against Malaria, can also be attributed to more than artemisinin. Ferreira et al. (2010) reported about flavonoids of *A. annua*—casticin and artemetin.

In experimental studies, Elfawal et al. (2012) reported that preparations from whole plants of *A. annua* may be more active than artemisinin and its derivatives when used for treating malaria. This has been interpreted as evidence of interactions or synergistic effects of plant constituents.

Czechowski et al. (2019) also examined the contribution of flavonoids and artemisinin in whole leaves, highlighting that whole leaves of *A. annua* should be taken into consideration instead of the single compound.

2.10.2 Anticancer Activity

During the last many years, the ability of *A. annua* and its constituents to inhibit cancer has gained increasing scientific interest. *A. annua* constituents were studied for biological activity by

different groups in relation to cancer models (Emami et al., 2010).

Artemisinin and some of its analogues have been tested for cytotoxic activity against various types of malignant cells. Mechanisms involving the endoperoxide bridge of artemisinin involved in interaction with different mechanisms that generate the reactive oxygen species (ROS) were discussed by Wang et al. (2015). These reactive species can be responsible for cellular damages and apoptosis during their action.

Chen et al. (2020) were also interested in the anti-cancer effects of artemisinin derivatives. However, other plant components of *A. annua* could also be responsible for anticancer action. Liu et al. (2016) examined coupling compound scopoletin and found it to have activity associated with inhibition of cancer-cell proliferation.

While these results indicate that the compound may have anticancer properties, there isn't a lot of conclusive evidence available yet. However, additional in vivo and clinical research is necessary to make this transition to defined therapeutic uses.

2.10.3 Antiparasitic Activity

The antiparasitic properties of *A. annua* are not limited to malaria. Artemisinin and its derivatives have been studied concerning a few protozoan parasites. In vitro activity of artemisinin against a protozoan parasite *Neospora caninum* (associated with neosporosis) was reported by Kim et al. 2002.

However, Artemisinin analogues are similarly being explored with respect to their enhanced antiparasitic activity. Arthritis was reported as being inhibited by artemisone and related compounds in experimental models, by Mazuz et al. (2012). Based on these results, it can be inferred that compounds related to Artemisinin have more wide-range antiparasitic activity.

Efferth & Oesch (2017) published a recent review of the action of artemisinin and derivatives on protozoa such as *Giardia* and *Leishmania*. Their analysis suggests there's more to the biological activity of artemisinin-like molecules than the benefits seen by treating cancer-causing parasites in malaria.

2.10.4 Gastrointestinal and Digestive Applications

A. annua has been used traditionally for gastrointestinal complaints and for appetite. The digestive uses of the plant have been linked with its antioxidant and anti-inflammatory effects.

In 2011, Zhou et al. looked into polyphenolics derived from plants for "antioxidant and gastrointestinal effects. There is however a mismatch between the evidence for digestives of *A. annua* as a function of the experimental organism and preparation. Therefore caution should be exercised when interpreting traditional uses as separate from pharmacological activity that has been demonstrated.

2.10.5 Antimicrobial Activity

Biological properties of *A. annua* are of the highest relevance for the present work and account for its antimicrobial activity. Antimicrobial properties of essential oils derived from plants have been shown by Takarada et al. (2004) with some bacterial species.

Ahmad Radulovic et al. (2006) studied the antibacterial and antioxidant properties of essential oil from *A. annua*. They reported that they identified that EO has inhibitory activity against selected microorganisms with varying susceptibility of the different microbial species.

This difference in antimicrobial susceptibility could be due to differences in microbial cell-wall structure, membrane composition, and cell permeability and interactions with distinct components of the essential oils. Thus, the antimicrobial activity of the oil needs to be interpreted according to its chemical characterization.

2.10.6 Anti-inflammatory Activity

Among the various classes of secondary metabolites, the anti-inflammatory activity of *A. annua* has been studied. Some of the compounds in the plant include flavonoids, coumarins, terpenoids and others that may help in the anti-inflammatory process.

Many secondary metabolites are present in *A. annua* with biological activities, which has been

under reviewed by Bakshi et al. (2001) and highlighted there that many different kinds of compounds are related to the pharmacological activities. Its constituents, namely scopolin, artemisinin and derivatives have been studied as anti-inflammatory agents.

Selected Swedish medicinal plants were investigated by Tunon et al. (2006) and added into the overall body of evidence for the biological properties of *Artemisia* medicinal plants.

2.10.7 Antioxidant Activity

Antioxidant activity has also been reported for *A. annua*. Ferreira et al. (2010) reported the occurrence of flavonoids amongst which C-glycosyl flavonoids in the aerial parts of the plant. Flavonoids could be responsible for antioxidant effects by scavenging free radicals and by regulating and affecting oxidative processes. A number of other constituents, such as the essential oils, could also be responsible for the antioxidant activity of plant preparations. Thus, the antioxidant activity of *A. annua* is not solely due to a constituent but to a combined chemical composition.

2.10.8 Antiviral Activity

Antiviral activity has been reported for *A. annua* preparations. We followed the work of Lubbe et al. (2012) who studied the anti HIV activity of *A. annua* infusion in vitro at low concentrations. ARTs/artemisins may exert some activity aside from the structural component they add to the plants, their findings indicated.

The finding of different activities of whole-plant preparations when compared with purified artemisinin, highlights the need to investigate the complete phytochemical profile of medicinal plants. Data regarding in vitro antiviral activity, however, should not be considered an indication of clinical efficacy, until proper clinical investigation is performed.

2.10.9 Antibacterial and Antifungal Activity of Essential Oil

A. annua essential oil exhibited antibacterial and antifungal activity which is significant in the investigations related to GC-MS characterization. Juteau et al. (2003) mentioned *A. annua* essential oil has antibacterial and antioxidant effect.

Shawish and colleagues (2014) studied the antibacterial properties of extracts from *A. annua* against the bacterial model pathogen *Escherichia coli*, and found that the properties of these extracts were dependent on the origin and extraction solvent used for the plants. The results of this study indicate that different extraction parameters and geographical origin can affect the biological activity.

Rosa et al (2015) evaluated *A. annua* essential oil (EO) and key phytochemicals camphor and artemisia ketone against food micro-organisms including *Yersinia enterocolitica*, *Listeria monocytogenes* and *E. coli* O157. The essential oil and main compound were inhibitory towards the tested microorganisms.

Rosa et al. (2015) put forward the assumption that the constituents of essential-oils interact with microbial membranes and lead to higher permeability of membranes, causing ions and intracellular parts to leak out. These effects may lead to anomalies in cellular processes, adding to the inhibition of microorganisms.

Some studies recently performed still confirm *A. annua* essential oil as a source of antimicrobial compounds. This is reaffirming the value of chemical profiling coupled to biological activity as 37 constituents were identified in *A. annua* essential oil and its antioxidant and antimicrobial activities explored in an upcoming GC-MS study (2025).

2.10.10 Activity Against Other Protozoa

Artemisinin-related compounds have demonstrated activity against protozoan parasites other than *Plasmodium*. Efferth and Oesch (2017) researched the anti-organic activity of broad spectrum and found the presence of

research drawback against *Leishmania* and *Giardia*.

Other herbs used in phthalic and veterinary medicine—such as those from *A. annua*—have been tested against *Acanthamoeba*. Different extracts of *A. annua* were found to be active against *Acanthamoeba* species by Derda et al. (2016). The study showed that some variations exist in terms of activities depending upon extraction solvent and preparation procedure.

It shows that the biological activity of *A. annua* is wide and the chemical compositions present in various formulations could radically affect their activity.

2.11 Traditional Uses of *Artemisia annua*

The traditional medicinal use of *A. annua* is particularly well documented in Asian medicine. Hsu (2006) traced back the Chinese materia medica history on the uses of qinghao, and explained that the species has long been important in traditional Chinese medicine.

All parts of the plant have been used in herbal medicine for several ailments, such as flowers, leaves, important oils, essential oils, seeds and stems. Kumar et al. (2020) found fever, infections and jaundice as the traditional applications.

The traditional applications of *A. annua* against fever, haemorrhoid and wounds were described by Li & Wang (2019). Wang and Li (2015) reported that dried plant materials are traditionally prepared as tincture, infusions or water soluble extracts.

A. annua have been traditionally used for infectious and parasitic diseases, which is discussed by Chen et al. (2018). Arkanglia et al. (2017) noted traditional application of seed preparations in the treatment of some eye diseases.

For modern pharmaceutical or biomedical studies there is a compound interest, in traditional usage, throughout a long history of practical use. Active compounds have a very long history of traditional use, based on which today, pharmacy or biomedical research is carried out. Avoid interpretation of traditional applications as evidence of clinical efficacy, however, traditional

applications should be handled in conjunction with experimental.

2.12 Contemporary Phytotherapy and Global Medicinal Importance

Modern research has expanded the potential applications of *A. annua* beyond its traditional uses. It has been studied for producing artemisinin, essential oils, flavonoids, terpenoids and other biologically active components.

Artemisia species have been studied by Wright and Linley (2010) as therapeutic plants and they emphasized the possibility of chemical constituents found in the different species. Similarly, Bisht et al., (2021) had conducted a review on the phytochemistry and pharmacological applications of *Artemisia* species and reported a plethora of biological studies.

The essential oil is of special interest due to its complex chemical structure and biological activity. The study conducted by Donato et al. (2015) validates the possibility of using the essential oil and the main components of *A. annua* as biological and food-related ingredients, due to their efficacy against foodborne microorganisms.

The present studies reveal that *A. annua* has continued scientific interest and clearly indicate the need for supplementing traditional knowledge with contemporary analytical chemistry and biological assessment.

2.13 Applications in Cosmetology

The chemical properties of *A. annua* have also resulted in interest in cosmetic applications. According to Szopa et al. (2020), cosmetics uses of *A. annua* contain ingredients that have antioxidant, antimicrobial, scent, softening, and conditioning properties.

The volatile constituents and biological properties of plant extracts and essential oils can make them useful in cosmetic products. But the safety and efficacy of the individual preparations may vary depending on their chemical constituents, level, stability and application method.

The commercial potential of *A. annua* in cosmetics is well illustrated but is not as a main priority for the present research programming focusing on GC-MS characterization and biological evaluation.

2.14 Food and Culinary Applications

A. annua has also been associated with food-related applications. Hsu reported traditional uses of the plant from China (2006) and Liu et al. reported usage for applications using a plant material as a natural green colorant and flavoring component (2010).

The following are some of the applications used to prove that *A. annua* can be utilized in the field other than medicines. However, these nutritional/culinary uses are not more directly related to the aims of the present investigation and are thus used as supplemental background.

2.15 GC-MS Analysis of *Artemisia annua* Essential Oil

Gas chromatography-mass spectrometry (GC-MS) is an analytical technique that is useful in the characterization of complex mixture of volatile compounds. GC gives the chromatographic separation of volatiles, MS gives the mass-spectral information, which helps to identify the volatiles. This pairing is therefore that most suitable pairing for essential oils.

The information about retention time, RI, molecular mass, the mass spectrum characteristics and relative amount of each component can be obtained from the GC-MS analysis. The data may be used in forming a 'chemical fingerprint' of an essential oil.

In the case of *A. annua*, the essential oil contains a large number of oxygenated compounds, sesquiterpenes, and even monoterpenes that make this species of interest to be analyzed by GC-MS. The recent literature proves that GC-MS-based investigation may determine dozens of components in *A. annua* essential oil and it can be used with antimicrobial and antioxidant tests. Chemical composition of *A. annua* essential oil varies in the different studies. This variation could be due to: geographical origin, genotype,

growing environment, maturity, plant part, and extraction process. Hence, GC-MS profiling of a well-defined plant population is beneficial to record its chemistry.

2.16 Essential-Oil Composition and Chemotaxonomic Relevance

The chemical composition of essential oils can provide useful information for chemotaxonomic studies. Similar species may have many of the same constituents while other differences in abundant compounds may differentiate a species, population, and/or chemotype.

Abad et al. 2012 showed high chemical variability of *Artemisia* essential oils. Jaitak et al. (2008) showed that the essential-oil composition of *A. maritima* varies among various locations. Also, a variation in the volatile-oil constituents of *A. scoparia* from various growing areas was reported by Lin et al. (2004).

From these results, it is concluded that the geographical and environmental data must be taken into account for the interpretation of essential-oil profiles. This chemical profile is representative of one population, but may not be truly representative of the entire species. Hence, if only one population or a single collection site is sampled, this term should be used with caution, namely there is only a chemospecific description of the collection site.

From the present study, the Chemical Fingerprint of the investigated *A. annua* is conducted by performing the GC-MS analysis. The identification of the major and minor constituents enables to compare with all published profiles and give an indication of the relative amount of certain terpenoids and other volatiles.

2.17 Relationship Between Chemical Composition and Biological Activity

The biological properties of an essential oil are closely related to its chemical composition. Some individual components of an essential oil are found to be antimicrobial, and the activity of a blend may be due to an interaction between more than one anti-compound.

Essential oil of *A. annua* has been reported as antimicrobial and is believed to be responsible for its activities via some compounds such as camphor and artemisia ketone (Rosa et al., 2015). These are able to disrupt cell membranes amongst other impacts on microbial cells.

Thus, the differences experienced in antimicrobial activity among essential oils can be explained by their chemical composition. Different proportions of major constituents mean different biological activities if populations of *A. annua* are in two different environments. It makes a compelling case for using GC-MS characterization in conjunction with antimicrobial testing.

The thesis is an example of this. In the essential oil of *A. annua* investigated 28 compounds exceeding 0.1% concentration were detected, at which is ca. 95.5% of the detected composition. The major constituents that were reported were: artemisia ketone (26.14%), β -caryophyllene (24.70%), camphor (10.66%), eucalyptol (8.05%) and other.

The thesis further reveals the antimicrobial activity against some of the tested microorganisms such as *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus mirabilis* and *Candida albicans*. In the light of the above results, it was decided to investigate if the chemical constituents identified by the GC-MS are related to the observed biological activity.

2.18 Research Gap and Rationale for the Present Study

Although *A. annua* has been extensively investigated for its medicinal importance and artemisinin content, its essential-oil composition can vary substantially according to geographical and environmental conditions. Essential oils have already been described in several populations but there can be no chemical identity between regions.

Moreover, most of the published report has been about artemisinin and antimalarial activity, whereas the nature–activity relationship of the full volatile chemical profile with antimicrobial

activity remains to be studied. Multiple constituents can be characterized at one time and a chemical fingerprint for a specific population of plants can be developed using modern GC-MS analysis.

In the present study therefore, emphasis was laid on the chemical characterization of essential oil by GC-MS analysis with the associated biological evaluation of *A. annua*. The strategy combines/combines the chemical profiling approach and the complementary FTIR characterization and the antimicrobial testing. According to the thesis, 28 compounds were identified using the Gas Chromatography with Mass Spectroscopy (GC-MS) and the composition of the essential-oil had a high proportion of these, about 95.5%.

Particularly important for chemotaxonomic studies is the chemical and biological integration as it enables the evaluation of the chemical properties of the plant material under investigation based on the biological properties. The resulting profile might be helpful for comparisons for future studies of *A. annua* from various geographic areas.

This literature review concluded that *A. annua* is a chemoparmacologically important and chemically diverse medicinal plant. The essential oil of its possesses a number of volatile components which have biological activity, and there are variations in chemical composition among species and populations, making it a basis for chemotaxonomic study. The use of GC-MS is thus a suitable analytical tool for the elucidation of the volatiles of *A. annua* and its possible association with biological activity.

3. Materials and Methods

3.1 Study Site and Experimental Materials

This study was performed under the supervision of Dr. Shah Iram Niaz at Natural Products Laboratory, Institute of Chemical Sciences. The plant material of *Artemisia annua* L. was collected from Mansehra (Pakistan) and the rest of the investigation was carried out there. This was done in the plants that were cultivated in Abbottabad.

The laboratory glassware that was used in the experimental work consisted of beakers, Erlenmeyer flasks, round bottom flasks, pipettes, graduated cylinders, test tubes, condensing flasks, separating funnels and volumetric flasks. The major equipment consisted of a heating mantle, water condenser, Clevenger-type apparatus, electronic balance and a round-bottom flask.

Water (dionized) was used in the preparation and extraction of aqueous materials. Analytical-grade ethanol (95%; Fisher Scientific) was used as an organic solvent and for preparation of a 70% ethanol solution for laboratory cleaning. If necessary for chromatography and biological preparations, HPLC grade methanol (Sigma-Aldrich) was used. The solvents used for glassware cleaning and dissolving selected compounds were analytical grade of acetone, Merck, and reagent grade of hexane, Fisher Scientific, also as diluent of the essential oil before the GC-MS analysis.

3.2 Plant Collection and Identification

Plant material of *Artemisia annua* L. was gathered from Mansehra, Pakistan, raw. Fresh raw *Artemisia annua* L. plant material was collected from Mansehra, Pakistan. The plant materials were studied based on the morphological traits especially leaf shape and structural features. The identification of the plants was done by the Herbarium section of Gomal University. It was additionally confirmed by the Gomal University Botanist Dr. Zain ul Abidin.

The collected plant material was transported to the laboratory where it was processed for the extraction of the essential oil.

3.3 Extraction of Essential Oil by Hydro-Distillation

The plant material of *A. annua* was subjected to hydro-distillation method for the extraction of essential oil by using Clevenger apparatus. The plants collected were chopped into small pieces and was put in the proper amount of water into the round bottom flask. The flask was attached to the Clevenger apparatus and condenser and was mounted on top of a heating mantle.

The mixture was gradually heated until boiling. In the hydro-distillation process the plant material was subjected to the steam produced by the water in the process and these volatiles ended up in the vapor phase. Volatile oil components in the steam then flowed through the condenser where they condensed back to a liquid form, containing water and essential oil.

The condensed distillate was collected in graduated arm of the Clevenger apparatus. In view of the density difference between water and essential oil, the essential oil separated from the water phase and was collected. In continuous distillation, the water was condensed back into the boiling flask which had not completely evaporated.

The essential oil recovered was diluted into a dark, tightly covered container and kept away from excess heat, air and light until further investigation.

3.4 GC-MS Analysis of Essential Oil

The chemical composition of the *A. annua* essential oil was determined by gas chromatography-mass spectrometry (GC-MS). A Shimadzu QP5050A gas chromatograph equipped with quadrupole mass spectrometer was used for analysis.

Prior to GC-MS analysis, the essential oil was diluted with hexane at a ratio of 1:10 (v/v). The diluted sample (1 μ L) was injected into the GC-MS system by split entry. A 1.5 mL/min supply of helium, 99.999% pure, was used as the carrier gas. The injector temperature was kept at 250 °C, and the detector temperature reported as 300 °C. The inlet pressure was 2.07×10^5 Pa.

The pre-set ramp temperature was set at 40 °C/min to 260 °C, with a 5-min hold, and another 10 min was held at the indicated conditions. The temperature of the GC - MS interface was kept at 320 °C and the temperature of the ionization source / trap temperature was kept at 230 °C.

The mass spectra were obtained from mass to mass 50-400 for electron-impact ionization. Identification of volatile constituents was carried out by comparing their mass spectra with the reference spectra in the available spectral

libraries, comparing their retention indices with those derived from a homologous series of alkanes (C9–C24) and, when available, comparing them with authentic reference compounds.

Normalised peaks areas (without correction) were used for quantitative characterization as a percentage of the total peak area detected. Replicate analyses were performed in order to evaluate repeatability and consistency of the analytical results for each sample. The GC-MS methodology was performed according to the procedure of Zhao et al. (2019) with the conditions above.

3.5 Fourier Transform Infrared Spectroscopy

The sensographic analysis of the major functional groups of the *A. annua* essential oil has been achieved by Fourier transform infrared (FTIR) spectroscopy. Identification of characteristic infrared absorption bands for functional groups in the volatiles was done using FTIR spectrum.

The obtained spectral profile gave additional information to the GC-MS analysis, as the main chemical groups characteristics of the essential oil could be identified.

3.6 Evaluation of Antimicrobial Activity

Anti-microbial activity against selected bacterial and fungal microorganisms of essential oil of *A. annua* was determined by agar well diffusion and minimum inhibitory concentration (MIC) assay.

3.6.1 Agar Well Diffusion Assay

The agar well diffusion method was employed to evaluate the inhibitory effect of *A. annua* essential oil against the selected test microorganisms. Plates were made using the different microorganisms and a well was aseptically made on the agar containing medium.

Essential-oil samples were placed in the wells and the inoculated plates were cultured under the same conditions as mentioned for the different test microorganisms. After incubation, the diameter of zone of inhibition around each well was measured for the evaluation of the antimicrobial activity.

The diameter of the inhibition zone was measured in mm. The zone of inhibition larger the more inhibitory activity it exhibited under the experimental conditions.

3.7 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration assay was used as a quantitative measure of the antimicrobial activity of the essential oil. The minimum inhibitory concentration (MIC) of the test essential oils was considered as the concentration at which the growth of the microorganism was inhibited.

A number of essential-oil dilutions were made and then tested against standard microbial inocula, following the methodology that involves preferentially dilutions. Different concentrations of essential oils were prepared and tested against standard microbial inoculants based on a dilution method. The inoculated test preparations were cultured at the appropriate temperatures with respect to the test microorganisms. After the incubation, the growth of microorganisms was observed visually and the lowest concentration in which no growth was detected was reported as the MIC.

The MIC procedure was done following the dilution method reported by Oussalah et al. (2007).

3.8 Experimental Reproducibility and Data Analysis

GC-MS measurements were performed in triplicate to ensure analytical repeatability. Normalized chromatographic peak areas were used to determine the relative amount of the individual volatile compounds. Identified chemical names and relative percentage of compounds reported.

Antimicrobial activity of the essential oil was estimated by the inhibition zone diameter for antimicrobial assays and also the MIC was used to quantitatively evaluate the antimicrobial activity of the essential oil.

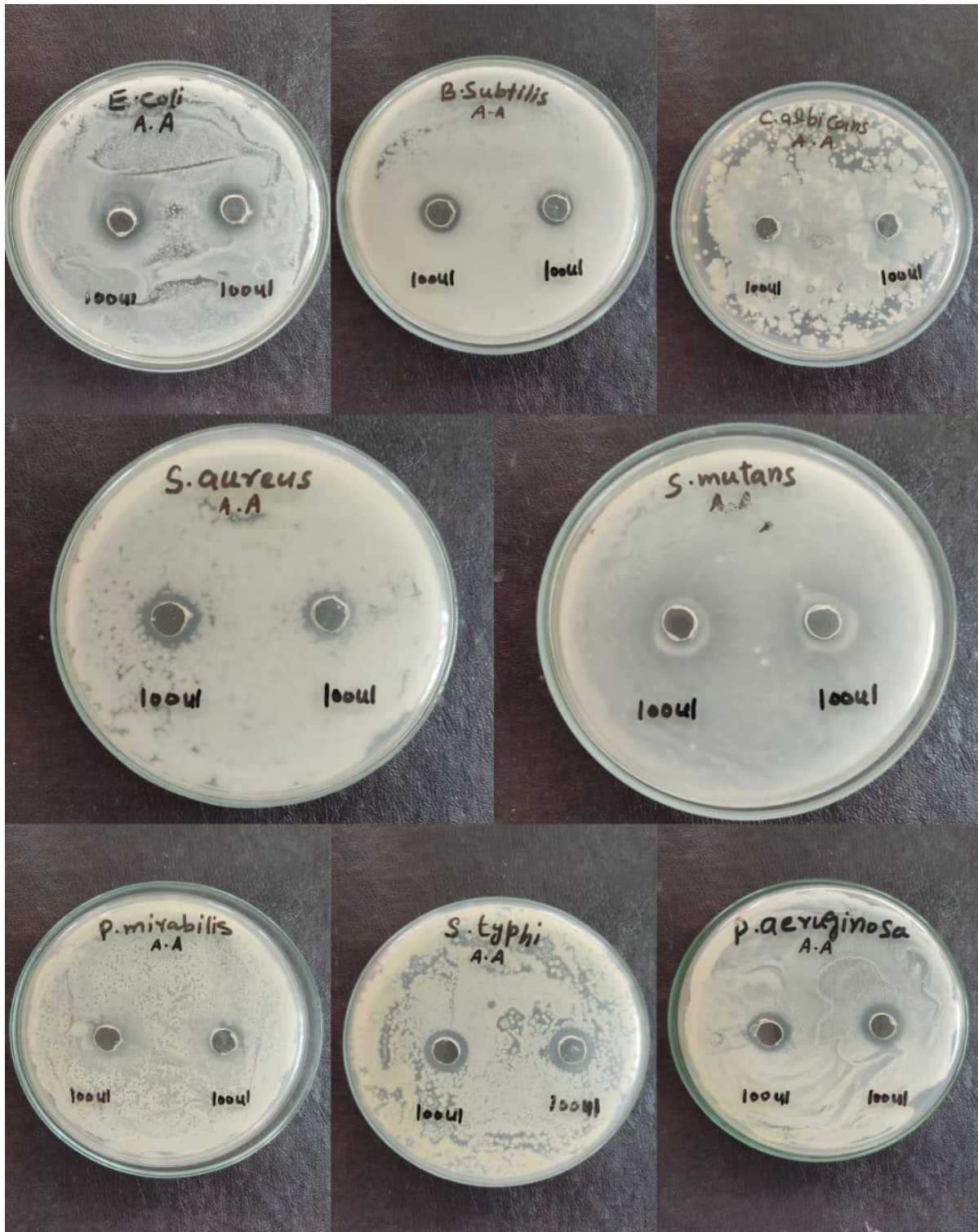
Results and Discussion**4.1 Result of Antimicrobial Activity**

Zone inhibition occurred after selected microbial strains were treated with *Artemisia Annua* oil for a whole day. When tested against bacteria, the

zone showed oil activity. When the oil content was 100%, the zone diameter, which was measured in millimeters, was determined by the essential oil concentration.

Table 4.1.1. Antimicrobial Activities of *Artemisia Annua*

SR.NO	Strain used	Zoon of inhibition mg/mL		Standard		
		Essential Oil		Azith	Capro	Clot
		R ₁	R ₂	(25µg)	(25µg)	(30µg)
1.	<i>S.typhi</i>	18	15	32	42	ND
2.	<i>P. aeruginosa</i>	15	16	39	37	ND
3.	<i>E.coli</i>	13	16	20	35	ND
4.	<i>S.aureus</i>	14	15	38	35	ND
5.	<i>B. subtilis</i>	13	14	26	44	ND
6.	<i>P.mirabilis</i>	10	10	41	42	ND
7.	<i>S.mutans</i>	0	0	30	48	ND
8.	<i>C. albicans</i>	12	13	31	49	33



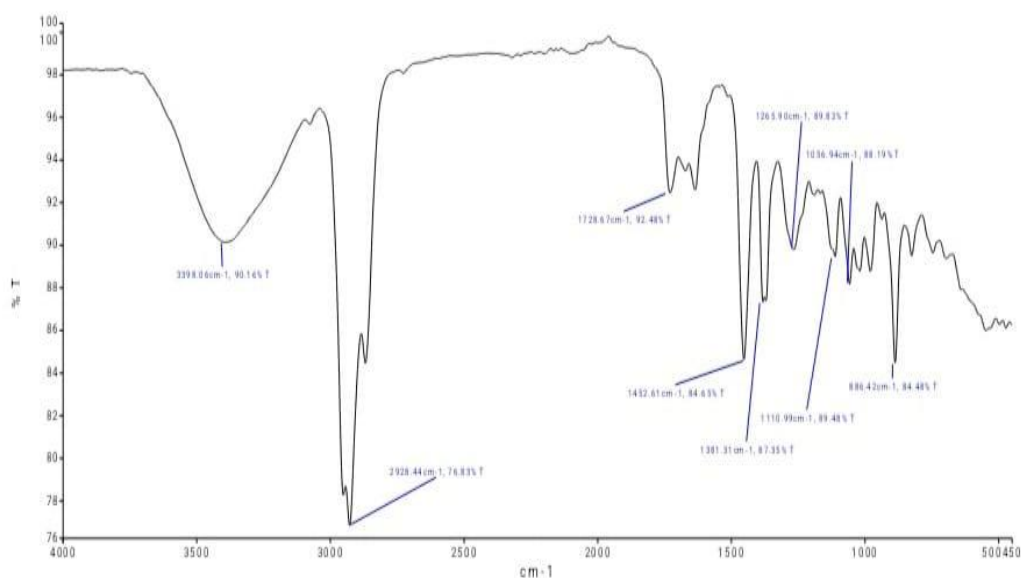
4.2 FTIR spectrum of *Artemisia Annua*

FTIR resulting spectrum sheds light on the molecular vibrations that distinguish different

types of chemical bonding. The absorption peaks found in the spectrum and compared to

established IR absorption ranges for functional

groups serve as the basis for the study.



Peak (cm ⁻¹)	Position	Transmittance %	Functional Group / Vibration	Typical (cm ⁻¹)	Range
3398.06		90.16	O-H stretch (alcohol or phenol, hydrogen bonded)	3200-3550	
2928.44		76.83	C-H stretch (alkane, sp ³)	2850-2960	
1728.67		92.48	C=O stretch (ester or carboxylic acid)	1700-1750	
1452.61		84.65	C-H bending (alkane)	1350-1470	
1381.31		87.35	CH ₃ symmetric bending	1365-1385	
1265.9		89.82	C-O stretch (ester or ether)	1000-1300	
1056.94		88.19	C-O-C stretch or C-O (alcohol/ether)	1050-1150	
1110.99		89.48	C-O stretching (alcohol, ester, ether)	1050-1300	
886.42		84.48	C-H bending (aromatic out-of-plane)	675-900	

FTIR Analysis of *Artemisia annua* Essential Oil

Essential oil obtained from *Artemisia annua* exhibited different absorption bands corresponding to different functional groups in its FTIR spectrum. A wide band of absorption at 3398cm⁻¹ was assigned to O-H stretching vibration. This is a broad band which can be a

sign of hydrogen bonding and may be indicative of the presence of hydroxyl containing species like alcohol or phenolics.

The presence of a strong absorption band at 2928 cm⁻¹ was interpreted as a C-H stretching one of aliphatic hydrocarbons with sp³ C atoms. This

band refers to the existence of the aliphatic structural components in the essential oils.

The presence of carbonyl-containing compounds was suggested by the potent absorption band at 1728 cm^{-1} which was assigned to the C=O stretching frequency. This absorption can be attributed to functionalities either as ester or carboxylic acids in the tested sample. The bands assigned to 1452 cm^{-1} and 1381 cm^{-1} were mostly due to C - H bending vibrations mostly related to methyl and methylene groups. The absorption near 886 cm^{-1} also could be related to bending vibration of hydrocarbon structural units.

Band at 1195, 1110, and 1056 cm^{-1} were also observed. The presence of these bands was indicative of C-O stretching vibrations and thus confirmed the presence of oxygen-functional groups such as an ester or ether linkage. The FTIR profile shows that the *A. annua* essential oil contains hydroxyl, aliphatic hydrocarbon, carbonyl and C-O containing functional groups. The information obtained from DT, FTIR confirms that obtained by the GC-MS analysis in a complementary way. Gas chromatography-mass spectrometry helps to separate and identify each individual component of the volatile one, whereas FTIR gives us information about the important groups of functional components which are present in the total essential oil sample. The results obtained by both analytical methods gave a wider picture of the chemical composition

of the essential oil oleoresin of *A. annua*.

Conclusion

The existence of hydroxyl (-OH), carbonyl (C=O), aliphatic C-H, and ether/ester functional groups is confirmed by the FTIR spectrum of sample Atms 2_1. The substance or mixture including alcohol derivatives or esters with potentially aromatic components is consistent with the combined peaks.

4.3GC-MS Analysis

Chemical composition and GCMS examination of *Artemisia Annua* essential oil during GCMS examination 28 compounds were detected in the essential oil of *Artemisia Annua*.

4.4 Chemical Composition of *Artemisia Annua* Essential Oil

The identification of twenty-eight components with concentrations greater than 0.1% accounted for 95.5% of all compounds found. The most prevalent class among them was oxygenated monoterpenes, which were followed by monoterpene hydrocarbons, phenylpropanoids, oxygenated sesquiterpenes, and hydrocarbons. The primary compounds, accounting for 65% of the makeup, were oxygenated terpenoids. Sesquiterpene hydrocarbons were the primary groups in the essential oil's structure, followed by monoterpene hydrocarbons and oxygenated monoterpenes.

Table 1. Chemical Composition *Artemisia Annua* Essential Oil

No	Component	Rel. % as determined by GC-MS	
		KI	Essential Oil
1	α -Pinene	940	1.51
2	Camphene	954	0.89
3	Sabinene	970	0.66
4	β -Pinene	973	0.51
5	β -Myrcene	989	1.04
6	2,5,5-Trimethyl-3,6-heptandiene-2-ol	1016	0.42
7	Eucalyptol	1026	8.05

8	Artemisia Ketone	1050	26.14
9	Artemisia alcohol	1066	3.01
10	Trans-pinocarveol	1134	1.19
11	Trans-verbenol	1139	0.61
12	Camphor	1147	10.66
13	Pinocarvone	1153	0.28
14	Terpinen-4-ol	1176	0.84
15	Eugenol	1357	3.32
16	β -Elemene	1388	0.60
17	α -Copaene	1374	0.37
18	β -Caryophyllene	1417	24.70
19	γ -Elemene	1432	0.20
20	α -Humulene	1452	0.20
21	Eugenyl acetate	1483	0.13
22	Germacrene D	1579	0.27
23	Caryophyllene oxide	1580	0.80
24	Arteannuin B	1690	3.67
25	n-Heptacosane	1698	0.29
26	n-Octacosane	1799	0.44
27	Arteannuic acid	1816	3.68
28	Lupenone	2854	1.02
Monoterpene hydrocarbons%			3.60
Oxygenated monoterpenes %			64.32
Sesquiterpene hydrocarbons %			3.01
Oxygenated sesquiterpenes %			5.70
Phenylpropanoids %			3.49
Hydrocarbons %			4.71
Other %			2.60

Total		95.5
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4.5 Major Compounds

According to the results, the extract had an average of three times the levels of the genotype-specific Artemisia ketone and Artemisia alcohol.

In addition, our product had three times as much camphor as eucalyptol and camphene combined. The primary ingredient in the essential oil was β -caryophyllene, whereas Artemisia ketone was the main ingredient.

Table 4.5 Major Compounds

No	Component	Rel. % as determined by GC-MS	
		KI	Essential Oil
1	Artemisia ketone	1050	26.14
2	β -Caryophyllene	1417	24.70

4.6 Structure of major compounds

Artemisia Ketone

β -caryophyllene

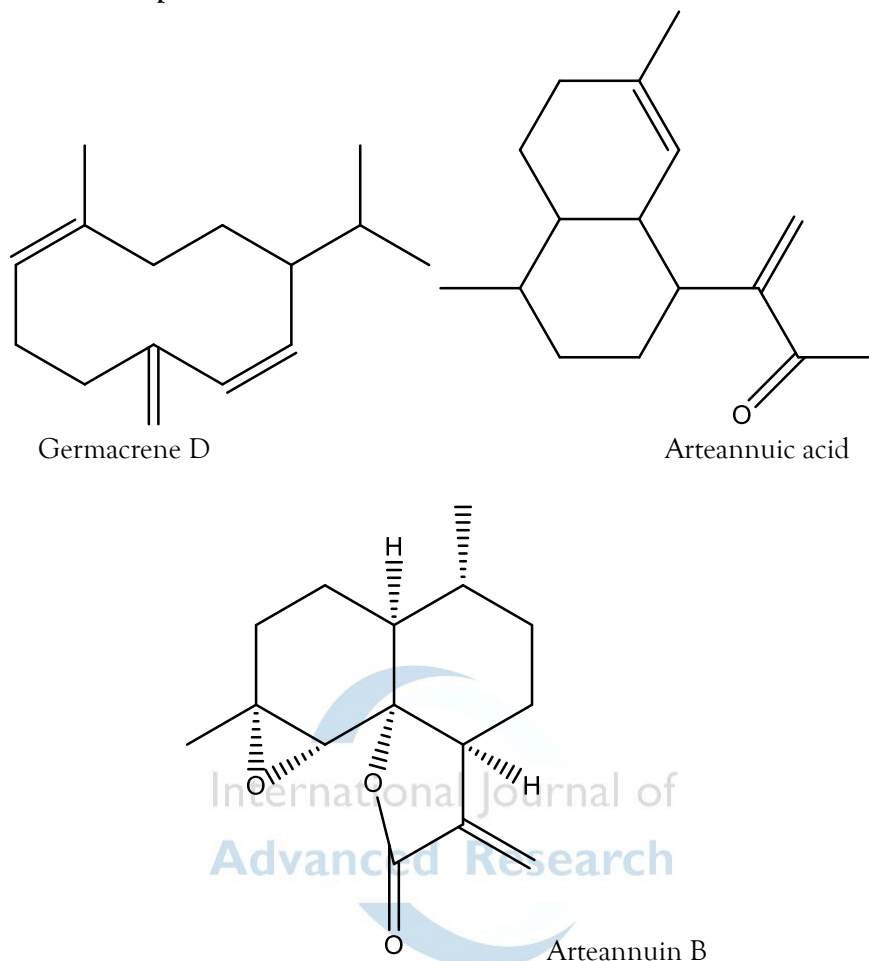
4.7 Minor Compounds

Arteannuin B, arteannuic acid, and the important germacrene D are all present in sufficient amounts.

Table 4.7 Minor Compounds

No	Component	Rel.% as determined by GC-MS	
		KI	Essential Oil
1	Germacrene D	1579	0.27
2	Arteannuin B	1690	3.67
3	Arteannuic acid	1816	3.68

4.8. Structure of minor compounds



4.7 Conclusion

The primary ingredient in the product's chemical composition is Artemisia ketone, which is primarily composed of oxygenated monoterpenes. Artemisinin and its derivatives, which have been shown to be present in sustainable amounts, are potential ant malarial components.

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