

# COMPREHENSIVE REVIEW OF *ACORUS TATARINOWII* RHIZOMA: MEDICINAL HISTORY, BOTANICAL SOURCES, GEOGRAPHICAL DISTRIBUTION, EXTRACTION AND ISOLATION METHODS, AND ANTI-MICROBIAL ACTIVITY

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## ABSTRACT

**Objective:** The dried rhizome of *Acorus tatarinowii* Schott., a perennial herb of the *Acoraceae* family, is known as *Acorus tatarinowii* Rhizoma (ATR), or Chinese Sweet Flag. The objective of this study is to determine the pharmacological potentiality, phytochemical constituents, and assessment of safety of ATR. It also aims for assessment of its conventional usage, therapeutical effectiveness and validation study for modernized therapeutical applications.

**Methods:** A systematically review of traditional literature and modernized scientific study was conducted. The analysis mainly highlighted the identification of bioactive phytochemical composition of ATR, which majorly includes presence of phenylpropanoid-groups, terpenoid-groups, lignan-groups, flavonoidal contents, alkaloidal contents and organic acids along with their therapeutical importance and pharmacological activities.

**Results:** Modern plant research has found more than 160 active ingredients, such as phenylpropanoids, terpenoids, lignans, flavonoids, alkaloids, and organic acids. These substances are responsible for helping with epilepsy, preventing seizures, fighting cell damage, calming the body, fighting cancer, and reducing swelling. Overall, *Acorus tatarinowii* is considered to be a healing plant which has been in use for a long time and even today, it interests the scientists and researchers.

**Conclusion:** ATR has also been used in clinical settings to treat neurological, cardiovascular, gastrointestinal, and respiratory problems, including epilepsy, depression, anxiety, and related cognitive deficits. Despite of treating various health disorders, still there are possibilities of various types of side effects caused by some of the main parts of the plant. This suggests that the amount of the plant part used must be controlled closely as well as its safety and efficacy must also be carefully tested.

**KEYWORDS:** *Acorus tatarinowii* rhizome (ATR), Medicinal biography, Extracting methods, Anti-microbial effect

## INTRODUCTION

The dehydrated part of the rhizome of *Acorus tatarinowii* Schott., a perennial grass belonging to the family of *Acoraceae*, is also referred to as *Acori Tatarinowii* Rhizoma (ATR) (Yan, L et al., 2020). It was earlier highlighted in Shennong's Classic book of Materia Medica of the medicinal plants, as a higher-ranking medicinal component for upgrading health and in chronic disorder treatment, ATR has been utilised in traditional Chinese medicine (TCM) for over 2000 years. According to Lam, K. Y. et al. (2016), ATR has an esteemed value in TCM due to its capacity to restore awareness, mind-relaxing, wetness-removal, and digestive-equilibrium assistance.

The ATR plant has been utilised in various clinical practices of China to cure disorders like respiratory illness, gastrointestinal diseases, heart disorders, and various types of neuronal diseases (Li, L. et al., 2018). The ancient and contemporary data emphasized its application for diseases such as epileptic disorders, depressive mind, anxiousness, lack of sleep, loss of memory, neuro-cognitive disorder, stroke, speechlessness, tinnitus, certain cancers, and skin disorders (Liu, L. et al., 2013; Lam, K. Y. C. et al., 2019). The evolving findings of newer pharmacological consequences and biologically active components have raised widespread interest in ATR plant. Current reports suggest its anti-epileptic properties, anti-convulsant properties, sedating properties, hypnotical properties, antioxidant properties, anti-cancerous properties, anti-tussive properties, and anti-asthmatic properties (Wu, J. et al., 2015; Lam, K. Y. C. et al., 2017; Shi, B. et al., 2020). These reports help in aiding this plant's potentiality as a cure for complex diseases like gastro-ulcers, depression, and neuro-cognitive disorder.

*Acorus tatarinowii* plant, is considered to be one of the most widely used species of the genus *Acorus*. It has its wide distribution across the north temperate areas to subtropical regions, especially in China, India, Japan, Korea, and Thailand (Wang, M. et al., 2023). The dehydrated rhizomes are the major medicinal elements and are ordinarily being used either alone or additionally with other medicinal herbs in ancient treatments for mind-related and neurocognitive disorders (Li, J. et al., 2021). Cleansed active portions serve as crude materials in the

pharmaceutical field, and commercially available Chinese patented medicines containing *A. tatarinowii* are widely being supplied in the market.

Phytonutrients analysis have identified over 160 essential components in *A. tatarinowii*, including various groups like phenylpropanoids, terpenoid-groups, lignans, flavonoid, alkaloidal groups, amides, and organic acid group (Wang, M. et al., 2022). These diversified portions contribute mainly to its expansive pharmacological roles. ATR is marked for its neuro-cognitive protective consequences, as it mediates the levels of neurotransmitters, which helps in increasing the blood flow to the brain, optimized blood flow to the cerebral part of brain, and also lessen damage to the neurons. These attributes offer therapeutical potentiality for mood disorders and neurodegenerative disorders. ATR is considered as one of the major medicines in several TCM-based therapy for neuro-cognitive deterioration and depressive state of mind.

Even though *A. tatarinowii* plant is prevalent in medications, current clinical studies suggest that few of its major components may cause uncertainty. Thorough risk evaluation and vigilant quantity control are essential for the plant. Forthcoming investigations should clearly classify the toxicodynamics, set up steady procedures for controlling the quality, and also safeguard the scientific usage

ATR is a remedial plant with chronicled and scientific importance. In spite of extensive exploration on its phytochemicals and pharmacological properties, much remains unfamiliar. A structured review of its ancient usage, phytochemical constituents, pharmacological properties, pharmacokinetics, and toxicological study is needed to direct forthcoming studies and aid in broader clinical usage. This review majorly aims in finding a systematic overview, identify research gaps, and inspire future research into the psychological mechanisms and medicinal potential of *Acorus tatarinowii* plant.

### **Therapeutical chronology of *A.tatarinowii* rhizoma plant (Shi-Chang-Pu)**

Previous documented study suggests that *Acorus tatarinowii* Rhizoma (ATR) has a curative chronology encompassing over 2000 years. ATR has strongly established in East-Asian medicinal practices, particularly in China, Korea, Japan, and South-eastern parts of Asia, where it was being included in both authorized recipes and ancient therapy. This plant also had its appearances in the famous Shen-Nong's Classic of Materia Medica record, where it has been considered as a first-class medicine for enhancing lifespan, secured long-lasting usage, and cognitive sharpness (Wen J et al., 2023).

Priorly available clinical documents of China have valued ATR for its odor and soothing appearance. Chronicle evidence also highlights its bitter taste, pungent smell and slightly warm characteristics, which, according to the Traditional Chinese Medicinal theory, make it more efficacious for resolving unpleasant dampness, unblocking sensorial orifices, and inducing a state of peace. Clinicians have used ATR for conditions linked to sputum, inaction, or unpleasant dampness that affect the brain and heart, often contributing to forgetfulness, disorientation, dizziness, and difficulty in speaking. These historical descriptions established ATR's main usage in enhancing resuscitation, soothing, and harmonizing metabolism, assisting its often incorporation in formulations for neuro-cognitive and cardio-vascular issues (Wen J et al., 2023).

Geographic disparities in the identification of the plant have complicated ATR's ancient records. Various species of the *Acorus* plants, including *A. gramineus*, *A. calamus*, and *A. tatarinowii*, were used exchangeability in different areas, affecting its terminology, commerce, and pharmacological status. In the twentieth century, advancement in pharmacognostical and taxonomical has led China and other higher organizations to clarify ATR plant's phyto-resource. By the mid-twentieth century, *Acorus tatarinowii* was recognized in official drug compendia book as the main source for Shi-Chang-Pu in various regions of China, validating its usage in mandated procedures. The rhizome part of the plant was officially added to the Pharmacopoeial book of the People's Republic of China in the year of 1963 and still has it's inclusion in the 2020th edition, underlining its ongoing clinical usages (Wang, M. et al., 2023; Lam, K. Y. et al., 2016).

Medicinal literary works have conserved both anecdotes and formulas that have influenced rituals. Some scripts linked ATR to procedures for neuro-cognitive enhancement, increasing alert behaviour in rituals, and fostering longevity, showcasing its communal, socio-cultural, and therapeutical importance. Progressively, ATR swapped from single-flora usage to incorporation in various medicinal recipes. Ancient usage has combined ATR with other medicinal plants to address post-stroke effects, neuronal-cognitive impairment symptoms, epileptic seizures, and listening or talking disability (Wen J et al., 2023).

Investigators have determined ATR's phytochemistry, while giving more emphasis on various types of volatile oils like beta-asarone and alpha-asarone as one of the key active phytoconstituents, and also bridged its pharmacological effects to determine bio-chemical and physiological effects. The modernized period, marked by the twentieth-century inclusion of TCM into governmental pharmacopeias, brought a technical review of ATR's traditional usage. The current studies have systematically combined ancient insights with scientific and pre-scientific data, showcasing the various traditional uses, particularly for neuronal-cognitive dysfunction, epileptic seizures, and bipolar diseases, are supported by neuronal-protective, anti-inflammatory, and neurotransmitter-regulating effects, which has been observed in-vitro and in-animal studies (Song, Z. et al., 2018).

Ethnobotanical study reports and ancient texts have continued to shape therapeutic assumptions and guide formulation, while modernized phytochemical profiles, pharmacological studies, and molecular verifications provide supporting evidence. Currently, ATR's role reflects an ongoing dialogue between science and culture. Recent systematical reviews have included this history, figuring out ATR's profile from its classical origins through regional uses, early pharmacopoeial acknowledgement, modernized herbal clarification, and modernized

pharmaco-toxicological research. This inclusion perspective is of great value for clinicians, researchers, and officials (Song, Z. et al., 2018).

In summary, the therapeutical usage of *Acorus tatarinowii* rhizome demonstrates abiding socio-cultural importance, clinical adaptability in TCM, and enhanced therapeutic validation and standardization. Once praised as an enhanced plant in ancient texts, ATR now serves as an example of how ancient remedies evolved through taxonomical analysis, trade license, regulation, and lab-related research. Further studies continue to emphasize on practical questionnaires about safety, dosage and therapeutical application.

### **Regional distribution of *Acorus tatarinowii* rhizome**

Source of Botanical plants and plant-characteristics:

*Acorus tatarinowii* is a helophyte (wetland-loving) herb forming creeping-rhizomes and linear leave of linear shape arising from a robust, smell of underground stem. The rhizome is the therapeutical portion cultivated for processing, and it contains volatile oils (including asarones), phenylpropanoids, terpenoids and a variety of other phytochemical classes that strengthen traditional uses and modern pharmacological uses (Wang, M. et al., 2023; Wen J et al., 2023). Morphologically the species is characterized by a flattened, fragrant rhizome covered with fibrous roots, and a spadix-type inflorescence typical of the genus *Acorus* (Lam, K. Y. et al., 2016).

Native range and broad geographical distribution:

The majority of *Acorus tatarinowii*'s native range is eastern and southeast Asia. It is found naturally in China (mainly south of the Yellow River), Taiwan, Japan (main islands except Hokkaido), the Korean Peninsula (mainly southern regions), northeastern India, and parts of Southeast Asia, including Thailand, Vietnam, Laos, and Myanmar, according to several contemporary reviews and floristic accounts (Lam, K. Y. et al., 2016; Sokoloff, D. D. et al., 2023). As a result, the species' range covers a wide latitudinal gradient and consists of both long-standing cultivation sites connected to traditional medicine supply chains and wild populations.

Regional centers of cultivation and wild populations:

Members of the *Acorus* species complex are found in temperate to subtropical zones according to reputable botanical databases (such as Kew's Plants of the World Online) and current taxonomic classifications, which reflect the genus's penchant for marshy, riparian, and wetland environments (Zhao, Y. et al., 2023). Cultivation is frequently done in flooded plots or damp fields that resemble natural environments; wild populations are more prevalent in wetter lowland areas, river valleys, and paddy margins. Outside of China, *Acorus* rhizomes are used for medicinal and ceremonial purposes in local customs in Japan, South Korea, northeast India, and portions of Southeast Asia, where there are localized wild stands and cultivation (Chen, W. et al., 2024).

Habitat threats, conservation and genetic resources:

Recent research shows that habitat loss, over-collection, and agricultural the transformation of wetland ecosystems have reduced the native genetic diversity of *A. tatarinowii* in some places. To preserve wild genetic resources while satisfying medical need, conservation-focused research has suggested core germplasm banks, enhanced growing techniques, and germplasm collection (Qin, Q. et al., 2025). Because genetic and environmental variation might affect the production and concentration of bioactive components employed therapeutically, such assessments are crucial.

Research gaps and future directions:

Despite the extensive ethnobotanical and distributional knowledge regarding *A. tatarinowii*, there are still gaps in long-term genetic diversity monitoring, chemotype-specific distribution, and high-resolution mapping of wild populations. Understanding how geography influences phytochemistry and clinical potential will be enhanced by combining field surveys, GIS mapping, molecular barcoding, and chemoprofiling. Furthermore, increasing sustainable farming initiatives in traditional production areas might ease the strain on wild stands while guaranteeing reliable, authentic raw materials for both conventional use and contemporary research.

The rhizome part of *Acorus tatarinowii*, or Shi-Chang-Pu, has risen from apparent *Acorus* sp., which has its nativity in eastern and south-eastern parts of Asia. The valuable usage of medicinal part of ATR has a requirement of continued herbal verification, efforts of conservation as well as control of the quality, depending upon the geographical status. Majorly, the collection, growth and cultivations occur in central part and southern part of China as well in its neighboring countries. The ATR plant's current as well as ancient medicinal usage depends totally on the safety and pharmacological factors, geographical origin and species recognition as well as on methods of processing.

### ***Acorus tatarinowii* rhizoma extracting methods and compounds isolation : Overview**

*Acori tatarinowii* rhizoma (ATR) contains essential bioactive components like  $\alpha$ -asarone and  $\beta$ -asarone, oils having volatility, terpenoids, lignans, flavonoidal compounds, and other secondary metabolic components. These essential compounds have differences in their stability as well as polarity due to which the extracting methods and compound isolating methods be chosen in a careful way to maintain biological activity and provide clarification for clarity and purity. Pet-ether or hexane, which are non-polar solvents are used to extract lipophilic oils having volatility, medial solvents like ethyl-acetate is used to target phenylpropanoids and polarized solvents like water or butanol is used for extracting organic acids as well as glycosidic groups. The extracting technique of sequential solvent is used to separate components by polarity (Wen J et al., 2023; Wang, M. et al., 2023). The accelerated method of extracting solvents which mainly include pressurized liquid extraction (PLE), This method provides efficient extraction involving decreased solvent usage and is gradually adopted for control of quality of herbal drugs (Wang, M. et al., 2023). In current scenario, both modernized and ancient green extraction procedures have

been used to collect the bioactive phytoconstituents of ATR plant for determination of pharmacological and quality control investigations.

#### 1. Preparation of plant material:

Firstly, the rhizome will be collected and authenticated, after which they will be cleaned and grounded into powder for enhancement of its surface area. The moisture has to be controlled by drying the part at low temperature in order to preserve the oils' of volatility. The final powdered extract is to be stored in clean and dried containers to inhibit any kind of loss of aromaticity of the components as well as to minimize any kind of oxidation (Lam, K. Y. et al., 2016).

#### 2. Conventional solvent extraction methods:

Secondly, the solvent has to be extracted for obtaining phytoconstituents from ATR plant. Various solvents like methanol, ethanol are commonly being used for extracting phenylpropanoid compounds and flavonoid components. There are also other methods like, maceration, which is a simplified approach to extract thermolabile compounds. The method of soxhlation can be utilized to increase efficiency, but it also has chances of degrading heat-sensitive compounds (Azwanida N.N., 2015). Method of reflux extraction can also be frequently used, particularly in Chinese pharmacological investigations, where ethanol-water mixtures are used to duplicate to prepare traditional decoction and enhance yield.

#### 3. Extraction of volatile oils:

Similarly, for extraction of ATR's oils of volatility, hydro-distillation and steam distillation methods are considered to be the suitable ancient methods. By using the method of hydro-distillation, the powdered part of rhizome is boiled in piping hot water, after which the evaporated oil is liquefied and collected. On the contrary, steam distillation allows limited exposure of heat due to which the delicate aromatic compounds are preserved. The progressive technique of Microwave-assisted hydro-distillation, allows reduced time of extraction and increased yield of oils of volatility (Zhao, Y. et al., 2023).

A green alternative method involves supercritical carbon dioxide extraction for isolating the oils of essentiality. In this method, highly pressurized CO<sub>2</sub> is used for extracting constituents having no polarity and it helps in providing higher yield of asarones quantity as well as it also maintains integrity of the essential chemicals (Reverchon E., 1997).

#### 4. Modern assisted extraction technologies:

In order to increase the sustainable and efficient extraction process, advanced extracting technologies are utilized to obtain essential components from ATR. To shorten the time of extraction, enhanced solvent penetration method i.e. Ultrasound-assisted extraction (UAE) can be used. This UAE method has effectiveness towards flavonoidal-groups and phenylpropanoid-groups, where it is operational at lower temperature, in order to avoid degradation (Chemat, F. et al., 2017).

The polar solvents present within the plant tissues gets heated by Microwave-assisted extraction (MAE) technique, which helps in providing higher yield than regular reflux techniques. These MAE techniques provides faster extraction, and has been used for reduction in solvent usage and better replicability (Routray W., & Orsat V., 2012).

#### 5. Isolation and purification of active constituents:

After extracting the crude components, particular compounds are being isolated using techniques of chromatography. Sephadex or silica gel column chromatography is frequently being used for segmentation. High-pressure liquid chromatography (HPLC) method is used for purification and quantification of essential compounds like  $\alpha$ -asarone and  $\beta$ -asarone. Preparative HPLC is used for isolation of pure components for pharmacological evaluation. For profiling of ATR oils of volatility, gas chromatography–mass spectrometry (GC-MS) is considered to be the main tool, which also identify essential oil constituents, and differentiates chemotypes across regions. The method of liquid chromatography–mass spectrometry (LC-MS) helps in analyzing non-volatile constituents, which also includes lignans and flavonoids.

The highly modernized techniques like counter-current chromatographic method (CCC) and higher-speed counter-current chromatographic technique (HSCCC) have been used for isolating phenylpropanoid-group without any irreversibility of adsorption on the phases of solid, which helps in encouraging recovery of various sensitive components (Ito Y., 2005).

#### 6. Quality control considerations:

As per the data and records of European Medicine Agency [EMA], 2026, *A.tatarinowii rhizoma* may contain traces of harmful components, notably beta-asarones. The extracting and isolating methodology must therefore help in the assessment of safety. The standard organizations need to provide standard extracting procedures, analysis of marker for quantification, and limitations on hazardous components. Methods like High-performance liquid chromatographic method or Chemical fingerprinting GC-MS are essentially required to ensure consistency in the safety and quality of clinical usage.

Over the past two decades, research from crude extract screening to bioactivity-guided isolation of volatile oils and pure phenylpropanoids has clarified how ATR rhizome and its constituents act against bacteria, fungi, and other microorganisms. This summary presents key findings, methodological trends, and remaining knowledge gaps in antimicrobial research on *A. tatarinowii* rhizome (Wang, Z. J. et al 2020; Wang, M. et al., 2023).

#### 7. Early screening studies and spectrum of activity:

Initially, the screening of phytochemicals and agar-based antimicrobial evaluation has found that Acorus-

rhizome extracts, including the *A. tatarinowii* as well as its related species, can stop a range of Gram(+ve) and Gram(-ve) bacteria, microorganisms like yeasts, and filamentous fungi. Although studies related to its mechanism of action remains restricted. There are various evidences which suggests that the volatile phenylpropanoid-group can cause disruption in the cellular membranes, thereby causing leakage of intracellular contents of the cell, and can also lead to inhibition of major metabolic enzymes. In various species of fungi, especially in the *Candida albicans*, the oils from ATR and asarone-rich components can induce changes in the morphology, can also cause reduction in the formation of biofilms, it also has impaired synthesis of major component like ergosterol, and it can also compromise the integrity of cell membrane. For the bacterial species, the essential oils of ATR can cause disruption in the potentiality of cell membrane and in respiratory metabolism, resulting in the bacteriocidal effects at sufficient level of concentrations (Zhao, Y. et al., 2023). Clinical reports also suggest that by using ethanol, methanol, and aqueous extracts variability in activity can be seen depending upon the polarity of solvent and type of test organism (Zhao, Y. et al., 2023; Haran, P. et al., 2024). The oils obtained from both hydro-distillation and solvent-extraction can show antimicrobial zones of inhibition in disc-diffusion method or well-diffusion tests, which provides indication of the presence of diffusible antimicrobial components in both fractions of volatility and non-volatility (Qin, DP. et al., 2017).

#### 8. Volatile oil and asarone derivatives:

The fractions of oil of volatility, are rich in alpha-asarones and beta-asarones, and serves as a primary carrier for anti-microbial potential. Gas chromatography–mass spectrometry reports and antimicrobial evaluation results show that essential oils from Acorus-rhizomes can lead to inhibition of *Candida albicans*, other fungal species pathogens, and certain bacterial species. This type of activity can be correlated with the relative abundance of asarones and other phenylpropanoid groups (Wang et al., 2020; Qin, DP. et al., 2017).

#### 9. Comparative species research in *A. tatarinowii* versus *A. calamus*:

As the ancient usage has exploited certain Acorus species, so comparative antimicrobial evaluation can show better activity profiles. Research on *A. calamus*, has shown significant anti-bacterial and anti-fungal activity, due to high  $\beta$ -asarones content. These findings may support similar expectations for the rhizome part of *A. tatarinowii* to show similar effect (Al-Mijalli. et al., 2025). Therefore, careful botanical authenticity is required for determination of anti-microbial effect in order to avoid any conflict between pharmacology and species-specific chemotypes (Lam, Y. et al., 2016).

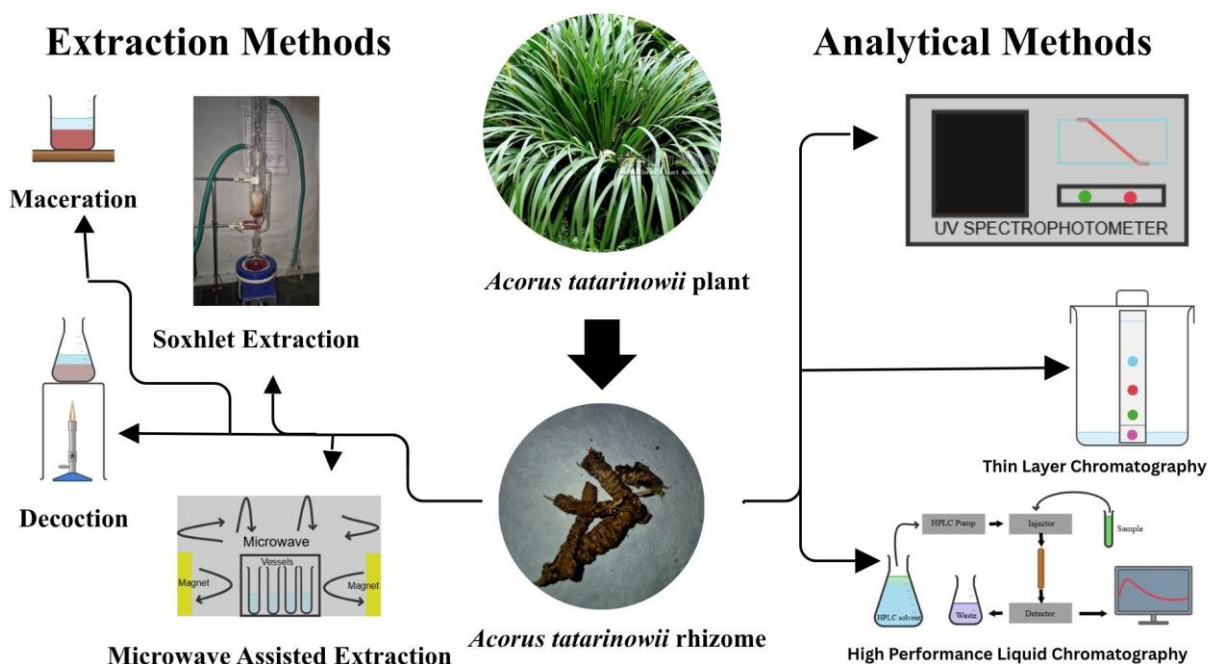


**Figure 1. Method of Extracting & Isolating Methods of ATR**

#### Determination of mechanism of ATR against microbes at molecular level

##### 1. Disruption of microbial cell membranes and permeability:

The microbial cell membrane disruption is one of the key mechanism shown by ATR plant. Fractions of essential oil and isolated isomers of asarones interjects into the bilayered lipid structures, thereby increasing the fluidity of the cell membrane as well as its permeability. This process can cause rapid leakage in the membrane by leakage of ions and small molecules (e.g.,  $K^+$ , ATP), collapse of potentiality of cell membrane, and loss of proton-motive strength. These series of episodes can rapidly cause disruption in the energy metabolism and can cause cell death in both bacterial and fungal species (Wang, Z J. et al., 2020). Transmission-electron microscopic method (TEM) and propidium-iodide uptake evaluation in several examinations showing extensive

damage to the cellular membrane after exposure to the fractions of oils of ATR plant, thereby promoting the primary membranolytic-mode (Qin, D P. et al., 2017).

## 2. Biosynthesis inhibition of ergosterol among fungal species:

In various species of fungal microorganisms, the volatile portions of ATR plant interfere with the metabolism of sterol. The result of bioassay has shown significant reduction in the content of ergosterol content as well as changes in the expression of genes of ergosterol formation in the treated cells of *Candida species*. The fungal cell membrane gets weakened due to the lowering of levels of ergosterol, which increases the cell's susceptibility towards osmotic-stress and oxidative-stress. These types of results mainly suggest that the essential components present in ATR mainly inhibit the pathway of synthesis of ergosterol by destruction of the redox balance of the cell, which helps in assembling the sterol groups (Zhao. Y. et al., 2023).

3. Stimulation of ROS: The rich components like phenylpropanoid groups present in ATR essentially helps in induction of oxidative stress intracellularly in the microbial organisms. There are certain in-vitro studies that have reported increased markers of Reactive-oxygen species (ROS), peroxidation of lipid groups, and oxidation of thiol groups after the treatment with essential components of ATR plant. These ROS are also associated with damage of DNA component, oxidation of proteins and disruption of the cell membrane integrity. The generation of ROS may result from components of respiratory-chains or from disturbances of cell membrane. The contributions from both of these mechanisms can lead to cell death through pathways of oxidation (Zhao. Y. et al., 2023).

4. Disruption of polymeric substances extracellularly and anti-biofilm activity: ATR plant's one of the key anti-microbial property is the inhibition of biofilms. Various reports have suggested that both isolation of phenylpropanoids and crude oils can help in prevention of formation of biofilms. These essential phytochemicals can also destroy biofilms of various species of *Candida* and bacterial pathogens. The biofilm inhibition can be supported by quantitative methods and con-focal microscopic techniques. The phytochemicals of ATR plant can help in reduction of surface adherence by alteration in the gene expression adhesion, increased permeability of cellular matrix, and by disruption of synthesis of matrix polysaccharides. This enhancement of cells allows co-administration of other drugs to allow penetration and act on the embedded cells (Zhao. Y. et al., 2023).

## 5. Interference with quorum sensing and virulence regulation:

The emerging reports suggest that the extracts of ATR plant can destroy quorum-sensing (QS) circuits, which helps in controlling the severity, maturation of biofilms, and systems for secretion in bacterial species. Clinical reports and transcriptomic studies suggest that the components of ATR plant downregulate the genes which are QS-controlled, decreasing the generation of severity factors like haemolysins and proteases, and can also help in decreasing the synthesis of toxins in pathogenic microorganism. ATR can lower pathogenicity and provide less pressure for resistance but detailed molecular mechanisms still remains unclear (Zhao. Y. et al., 2023).

## 6. Enzyme inhibition and metabolic interference:

In addition to the effects of cell membrane lysis, the phytochemicals of ATR significantly cause inhibition of microbial-enzymes, including the adenosine triphosphate synthase, respiratory enzymes, and metabolizing enzymes of lipids. In various fungal species, the portions of ATR can help in reduction of the activity of enzymes, which are essential for synthesis of cell wall and cell membrane. In various bacterial species, the profiling related to metabolomics helps in suggesting that the ATR phytochemicals can also inhibit the carbon metabolism as well as energy-generating enzymes, which causes impairment in the biosynthetic capacity and homeostasis (Qin, D P. et al., 2017).

## 7. Bonding with nucleic acids and proteins of microorganisms:

There are certain components of phenylpropanoid groups that have the capacity to bind with the proteins of bacterial species as well as fungal species, causing alteration in the structural and functionality of the enzymes. Processes like molecular docking and in-vitro binding evaluation methods suggest these groups interactions with ergosterol synthesized-enzymes, proteins of cellular membrane and binding proteins of nucleic acids, which have the potentiality to inhibit the replication, repair or transcription. Moreover, future confirmatory studies related to structure and biochemical processes are required to confirm these ligand-target. Certain phenylpropanoids and related compounds may bind to bacterial or fungal proteins, altering enzyme structure and function. Molecular docking and in vitro binding assays suggest interactions with membrane proteins, ergosterol biosynthetic enzymes, and nucleic acid-binding proteins, potentially inhibiting replication, transcription, or repair. However, confirmatory biochemical and structural studies are required to validate these ligand-target interactivity (Lam. Y. et al., 2016).

## 8. Limitations, toxicology considerations, and specificity:

According to the data and records of European Medical Agency [EMA], 2026, the systematic benefits of beta-asarones and phenylpropanoid groups is limited due to the toxicological concerns, as it may harm the human cells at high concentrations. Therefore, there should be dosage optimization with targeted drug delivery approach as well as there should be usage of chemo-types of lower-asarones to equalize the reduction in host toxicity and anti-microbial activity maintenance.

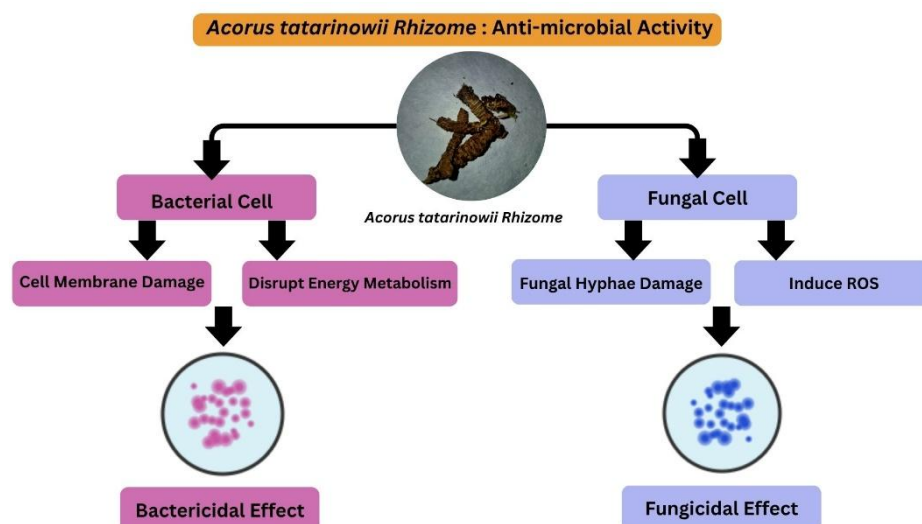


**Figure. 2. Mechanistic effect of ATR on microbial organisms**

### Toxicity testing profile of ATR plant:

#### 1. Acute and subacute toxicity:

The study for acute type of toxicity majorly measures the lethal dose for 50 percent individuals (LD50) as well as clinical symptoms after a single higher dosage. The aqueous and alcoholic extracts of ATR plant has demonstrated lower acute type of toxicity in standard mice models. For instance, when the aqueous extract is being given orally to the ICR mice, it had an LD50 value greater than 5,000 mg/kg, which indicated lower type of acute toxicity in the body (Liu. J. et al., 2024). The sub-acute toxicity study which was being conducted for a period of 28 days showed repeated dosing usage for identification of cumulative type of adverse effects. Many of the clinical reports have shown no significant changes in clinical usage at moderate type of dosage level. Therefore, there should be a careful selection of dosage level in chronic type of applications (Wen J. et al., 2023).

#### 2. Reproductive and developmental toxicity:

The clinical records related to toxicity in the reproductive system is very much limited, but higher exposing levels can pose a risk to the reproductive functions. Various studies on animal experimentation has indicated higher dosage of asarone group extracts, which may cause risk to the spermatogenesis, or may disrupt the estrogen cycle, or even slower growth of foetus. However, in some clinical trials, which are of controlled type, with usage of appropriate oral dosing, there were no adverse type of reproductive effects observed. Therefore, it is always recommended to neglect higher dosing of ATR components during pregnancy as well as in lactation (Wen J et al., 2023).

#### 3. Genotoxicity and mutagenicity:

As per the reports of in-vitro genotoxic testing, which mainly includes micro-nucleus testing, comet assay, ames test, the beta-asarones and its isomers may have a mutagenic or clastogenic effect after the metabolic activation. The reports from in-vitro hepatocyte testing and bacterial mutagenicity testing suggest that the isolating asarones group can have the potential to cause DNA damage, but still the data of in-vivo genotoxicity testing remains unpredictable. There are also certain studies related to micro-nucleus testing of bone marrow, which states that higher intraperitoneal dosages can have genotoxic effects. Similarly, certain reports also suggest that at lower dosage levels, upon contact with saliva, there is reduced or no genotoxicity observed (Wen J et al., 2023; Liu J et al., 2024).

#### 4. Chronic toxicity and carcinogenicity concerns:

The reports from certain chronic exposed studies raises concerns about carcinogenic effect of beta-asarones at higher exposure in rats tissues but still long-term study data remains limited. However, recent systematics reviews also suggest that though beta-asarones cause genotoxicity or carcinogenicity in some models, but the results for the whole plant of ATR being carcinogenic in the human beings remains indecisive as it mainly depends on the dosage levels, chemotypic characteristics, route of exposure (Uebel, T. et al., 2021).

#### 5. Organ-specific toxicity: hepatotoxicity, neurotoxicity, cardiotoxicity:

The metabolism from the hepatic route mainly influences the toxic effect and bioactivity of asarone groups. The beta-asarones gets metabolized to reactive molecules which may cause oxidative stress, peroxidation of lipid groups, and destruction of mitochondrial functions. As per the data of in-vitro hepatocyte testing, these processes can cause injury to the hepato-cellular activity (Patel, D. N. et al., 2015). Reports of neuro-toxicity testing remain unclear as some data suggests that certain isomeric molecules of asarones exhibit neuro-protective effect at lower dosage levels, whereas at higher dosage levels they can lead to abnormalities in behaviors in animal models (Chellian, R. et al., 2017). Similarly, the data of cardio-toxicity studies remains unclear and requires systematic safety evaluation.

#### 6. Processing, chemotype selection, and mitigation strategies:

Modernized processes like stir-frying, alcoholic extracting methods, and lower-asarone chemotype selection can reduce risk of toxicity, due to lowering of beta-asarones levels in the final formulated products. Similarly, by usage of supercritical carbon-dioxide extraction and selective fractionation techniques, there can be preservation of biologically active constituents with removal of toxic components. Therefore, there must be usage of pharmacopoeial standards and measure of control for quality, which must include specific limits for asarone usage as well as fingerprinting using chromatographic methods for safe clinical usage of these bio-active components (Wang, M. et al., 2023).

#### 7. Human exposure, reports and clinical safety:

The data from human safety clinical reports of ATR toxicological effect remains limited as compared to the pre-clinical studies. Earlier studies suggest that ATR levels are safer when used in consumable amounts. However, data of adverse drug reactions with usage of highly potent extracts remains unclear and thereby requires the need for pharmacovigilance. Therefore, there should be clear labeling and regulatory limits mentioned in the herbal products or extracts containing asarones as biologically active constituent.

### CONCLUSION

The medicinal herb *Acori Tatarinowii Rhizoma* (ATR) has a long-lasting therapeutical relevance, that serves as a connection between ancient knowledge and modernized scientific investigations. The Traditional Chinese Medicine has documentation of the usage of the ATR plant for over two thousand years in which it has been used as a herb to restore brain alertness, soothing action in the mind, de-humidification, and in regulation of digesting and systemic functionality. It has well established therapeutic effect in clinical usage in neurological disorder, gastrointestinal disorder, cardiovascular disorder, and respiratory disorder. Modernized pharmacological study reports further assist the traditional usages of ATR, which mainly depicts its anti-epileptic, anti-convulsant, anti-oxidant, anti-inflammatory, sedation, and neuro-protection effects.

There are certain investigations related to the phytochemical constituents of ATR which have demonstrated a diversified chemical profiling of ATR plant containing around one hundred sixty constituents, which majorly include phenylpropanoid groups, terpenoid groups, alkaloidal groups, flavonoidal groups as well as organic acids. Together, these components helps in treating complex type of diseases like depressive disorder, Alzheimer disease and other neuro-psychological disorders. The ATR plant has neuro-cognitive potential which is highlighted through its ability to regulate the neurotransmitters and thereby improving circulation to the cerebral systems.

Although, ATR plant has wide therapeutic potential in commercial usage, still it requires attentive application in clinical as well as toxicity studies due to the safety concerns regarding its biologically active components. There should be availability of standard toxicological investigations, regulation of dosage levels, and control of ATR's quality for its secured incorporation into biomedicines. The ATR plant remains a significant as well as a scientifically essential medicinal herb that guarantees continuing inter-disciplinary investigations to determine its mechanism of action, and curative properties optimization, which shall assist in evidence-based clinical usage.

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