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Exploring Galium Aparine as a Promising Natural Therapy for Oral Squamous Cell Carcinoma

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Abstract

Oral Squamous Cell Carcinoma (OSCC) is a head and neck cancer that has a detrimental impact on patients' quality of life. Surgery, radiation, and chemotherapy are standard treatment procedures that often have adverse effects and are limited in effectiveness, necessitating the quest for other therapeutic techniques.Galium aparine, a phytochemical, is currently acknowledged as a potentially effective treatment for OSCC. According to the literature, Galium aparine has a wide range of chemical elements, including flavonoids like quercetin and rutin, iridoids like asperuloside and aucubin, and phenolic acids like chlorogenic and caffeic acids. These chemicals have antioxidant, anticancer, and immunomodulatory properties, which make Galium aparine a potential natural treatment for OSCC.Preliminary investigations demonstrate its ability to inhibit cancer cell development in vitro and in vivo by inducing apoptosis and/or regulating immune responses. Furthermore, G.aparine extracts have been employed as hepatoprotective agents in a variety of malignancies, including breast carcinoma and melanoma.As a result of Galium aparineextracts's proven antioxidant and anti-cancer properties in cancerous tissues elsewhere in the body, we have finally concluded that it might possess similar properties in OSCC. To fully understand its modes of action and possible impact on improving outcomes for patients with oral squamous cell carcinoma, more investigation and clinical trials are needed.This paper provides an overview of Galium aparine's biological activities, composition, and role as a powerful anti-cancer agent, as well as potential future research directions.

Keywords: *Galium, Immunomodulatory Activities, Oral Squamous Cell Carcinoma, Tobacco.*

Introduction and Background

Overview of Oral Squamous Cell Carcinoma

Oral cancer develops from tissues in the throat or mouth. Among other parts of the oral cavity, it can impact the tonsils, floor of the mouth, roof of the mouth, lips, tongue, gums, and cheeks. Dysphagia, persistent mouth pain, vocal anomalies, or an unhealing growth or

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sore are some of the symptoms of oral cancer. Oral cancer can be treated with a variety of methods, including as immunotherapy, hormone treatment, chemotherapy, radiation therapy, and surgery.[1].

Epidemiology, Prevalence, and Current Treatment Challenges

As reported in 2020, the Global Cancer Observatory displayed 377,713 OSCC instances globally, while the most frequent ones were evident in Asia1. The risk factors for OSCC typically include tobacco products, alcohol, and viruses, depending on the population's lifestyles. Usu- ally, elements derived from tobacco smoking, such as nitrosamines and aromatic amines, significantly compromise the immune defences and elevate the chances of developing cancer in the mouth. Similarly, chewing tobacco products have also been significantly associated with an increased risk of oral cancer [2]. In 2007, the American Institute for Cancer Research (AIRC) confirmed that both cigarette smoke and smokeless tobacco products are carcinogenic as they generate nitrogen and oxygen free radicals and disrupt antioxidant defence mechanisms in the oral lining. Additionally, susceptibility to oral cancers also increases due to alcohol consumption through nitrosamine and mycotoxin-associated dysregulation. Drinking also enhances the carcinogenic effects of tobacco, creating a synergistic risk for upper aero-digestive tract cancers in individuals who both smoke and drink. This increased risk is due to the role of the enzyme cytochrome p450 (CYP2E1), which is activated by ethanol and helps metabolize the carcinogenic N-nitrosamines found in tobacco. Earlier studies have reported the high occurrences of viruses in oral cancer. OSCC-associated most common viruses include human papillomavirus (HPV), hepatitis C virus, herpes viruses and adenoviruses [2]. DNA viruses (HPVs) are found to infect epithelial cells and cause abnormal growths like warts and verrucous lesions in the skin and mucous membranes. There are nearly 100 different HPV types, but HPV-16 and HPV-18 are particularly concerning due to their strong association with cancer, earning them the designation of high-risk or oncogenic genotypes. There is no single known causal factor for oral squamous cell carcinoma; rather, it is a complex disease. The action of numerous internal and external elements is responsible for malignant transformation.[3].

In the initial phases, OSCC often does not cause pain, but as it advances, discomfort, abscesses, and ulcers become more severe [4], The back of the tongue is the most frequent location for OSCC, accounting for approximately half of all cases, followed by the floor of the mouth, soft palate, gums, inner cheeks, and hard palate. OSCC typically spreads to lymph nodes on the same side of the neck initially but can also involve nodes on both sides.

The risk of developing invasive oral cancers is higher for patients with oral potential malignant disorders (OPMDs) than for those with healthy oral mucosa [5]. Even though many individuals with OPMDs do not complain of any sickness when they are diagnosed, there may be signs that indicate the possibility of malignancy, such as redness, pain, tingling or ulceration[6].Therefore, healthcare providers must recognize OPMDs to establish OSCC's chances and the best management approach. These include conditions like OE (oral erythroplakia), OL (oral leukoplakia), OLP (oral lichen planus), and OSMF (submucous fibrosis) [7].

Despite new and improved diagnostic treatments, the overall OSCC survival rate is still around 50%[8]. Consequently, there are concerns about the limitations of conventional therapy. This has sparked an upsurge in research on other approaches for managing OSCC that are more tolerable and less toxic.

Introduction to *Galiumaparine*

Plant-based or synthetic derivatives (25-48%) are often used as alternatives to traditional medicine. One such derivative is *Galium aparine*, which belongs to the Rubiaceae family [9] and is known for its therapeutic properties in the treatment of wounds, lymph node swellings, epileptic conditions, hypertension and cancer [10]. The taxonomical classification is shown in Table1.*Galium aparine* contains active components such as flavonoids, quinones,alkanes, tannins, polyphenolics, and adequate levels of vitaminC [11]. Several in vitro studies have demonstrated that Galium species have anti-proliferative effects on leukaemia cells, breast cancer cells, laryngeal carcinoma, and HNC cell lines [12].

Table 1. Taxonomical Classification of *Galium aparine*[9]

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Rubiales
Family	Rubiaceae
Genus	*Galium*
Species	*aparine*

Phytochemical Composition and Biological Activities

Disturbances in the immune system can cause and worsen chronic diseases [13], [14]. Many studies have shown that restoring immune system function is essential for effectively treating various illnesses. The immune response is developed through the cooperative interaction of T cells, B cells, and macrophages, which involves the activation, cell division, and differentiation of these immune cells. Secondary metabolites derived from plant-based compounds comprising sterols, alkaloids, glycoproteins, flavonoids and lectins are used to modulate the immune system. For instance, specific polysaccharides like acidic arabinogalactan and rhamnogalacturonan have demonstrated immune-boosting properties in both laboratory and real-world settings[15]. Numerous studies have explored the immunomodulatory effects of saponins, with triterpenoid glycosides, in particular, showing significant benefits for the mammalian immune systems [16].

Previous experimentations have shown that commercial extracts from the plant *Galium verum* L contain immunology-modulating activity [17]. and has been detected in both aqueous and ethanol-soluble fractions, more specifically, about *Galium aparine* L. This species is known as cleavers or goosegrass and belongs to the Galium genus. The association is mainly apparent in Europe, Ukraine, some North America and in several Asian states including Alaska and Greenland. Furthermore, it is known as *Galium aparine* L.; non-native plant in some proportions of New Zealand, Australia and the sub-Antarctic Islands [18].

The detailed analysis of phytochemicals has shown that *G. aparine* contains iridoid derivatives, for instance, asperulosidic acid, asperuloside, acumine, monotropein, and aucubin[19]. It also contains alkaloids such as protopine, (−)-l-hydroxypeganine, (−)-8-hydroxy-2,3-dihydrodesox-ypeganine, and harmine[20]. Additionally, among the herbs, cleavers also possess hydroxycinnamic and

phenol-carbonic acids. Some of them can be enlisted as chlorogenic, caffeic, ferulic and gentisicacids [17], [21] They also contain flavonoids which include quercetins, rutin, hyperoside, kaempferol and its glucosides-astragalin, epicatechin, luteolin and apigenin [19], [21] The plant also contains several phytosterols among which campesterol, stigmasterol and sitosterol are the major compounds [22].

Phytochemicals are usually extracted from plants using a mix of alcohol or acetone with water, tailored to the specific compounds being targeted [23]. Techniques like HPLC, MS/MS, UH- PLC, and NMR are often used in these extraction processes [17], [23]. For example, Mitova et al. studied the iridoid patterns in Bulgarian Galium species, while Vlase et al.,analysed the polyphenolic content in Romanian Galium species [24]. The phytochemical content of a plant is greatly influenced by its geographic origin [25] and growth conditions, indicating that Galium species from different parts of Europe may have unique phytochemical profiles. This underscores the need to evaluate medicinal plants based on their growing environments. It's worth noting that Galium species from northern European countries haven't been extensively researched. Additionally, when considering plant extracts for therapeutic purposes, it's important to assess their antioxidant capacity. Antioxidants are crucial because they neutralize excess free radicals, which can damage cells and contribute to various diseases, thereby helping to prevent or reduce illness [26].

Biological Activities of *Galiumaparine*

Galium aparine is recognized for its diverse health benefits, including its antioxidant, anticancer, hepatoprotective, detoxifying, antihemolytic, antifungal, and antibacterial effects [27],[28]. Many studies on medicinal plants have highlighted the role of specific polyphenols and iridoids, which are abundant in various plant extracts, including those from the Galium genus [23], [24], [28]. Polyphenols, which are common secondary metabolites in plants, are particularly renowned for their potent antioxidant properties [23]. They also affect enzymes, and cellular receptors, counteract free radicals, reduce white blood cell immobilization, trigger cell death, control nitric oxide levels and hinder cell growth and the formation of new blood vessels [26], [29]. These diverse actions make polyphenols effective in both preventing and treating a wide range of diseases. Epidemiological research suggests that polyphenols can help lower the risk of cardiovascular diseases, neurodegenerative conditions, and cancers associated with oxidative stress [29], [30].

Plant phenolics encompass a broad array of compounds, including flavonoids, phenolic acids, tannins and lignans. Phenolic acids are further categorised into two types: derivatives of benzoic acid- Arabinonic acid, gallic acid and derivatives of cinnamic acid- ferulic acid, coumaric acid, caffeic acid [26]. Flavonoids, which are commonly found in various foods, offer numer- ous health benefits primarily due to their antioxidant properties and their ability to neutralise free radicals in the body. These benefits include liver protection, reduction of atherosclerosis risk, alleviationof inflammation, prevention of blood clot formation, cancer-fighting properties, support for bone health, and defence against bacterial and viral infections[30]. Some commonly encountered flavonoids include quercetin, catechin, rutin, cyanidin-glucoside, kaempferol and glycitein[29].

Iridoids, which are significant bioactive compounds, are present in various land plants andare mainly in the form of iridoid glycosides [31].Secoiridoids, which are derived from iridoids, can be found in approximately 57 different families of plants [21]. Different plant genera have specific chemical markers called iridoids. For example, Galium contains

asperuloside, Plantago contains aucubin, and Scrophularia contains aucubin and harpagide[19]. In traditional medicine, iridoids are commonly found in medicinal plants used to address various health issues such as diabetes, fever, wounds, skin problems, and inflammation. Research has shown that iridoids offer a wide array of health benefits, including protection for the nervous system, regulation of the immune system, management of diabetes, safeguarding the heart, liver, and gallbladder, as well as lowering blood sugar and lipids. Moreover, they demonstrate anti-inflammatory properties, promote wound healing, relieve spasms, and exhibit potential in fighting cancer, viruses, bacteria, and fungi [31].

Mechanism of Actions Against OSCC

The emerging dietary components and plant extracts are often considered promising alternative solutions. Despite advancements in treatment, chemotherapy often falls short, causing severe side effects like myelosuppression, hair loss, nausea, and vomiting. Moreover, many patients develop multidrug resistance (MDR), which accounts for about 90% of chemotherapyrelated cancer deaths, further diminishing its effectiveeness [32].

In light of these issues, researchers are turning to dietary factors for new anticancer strategies. These natural compounds offer diverse molecular properties and can trigger processes that kill cancer cells, potentially providing safer and more effective alternatives to conventional chemotherapy [33]. To understand the inhibitory mechanism of *Galium aparine* in oral squamous cell carcinoma (OSSC), the various signalling pathways involved must first be examined. The flowchart for the therapeutics of *Galium aparine* is shown in Figure1. The pathways are further discussed in the next section.

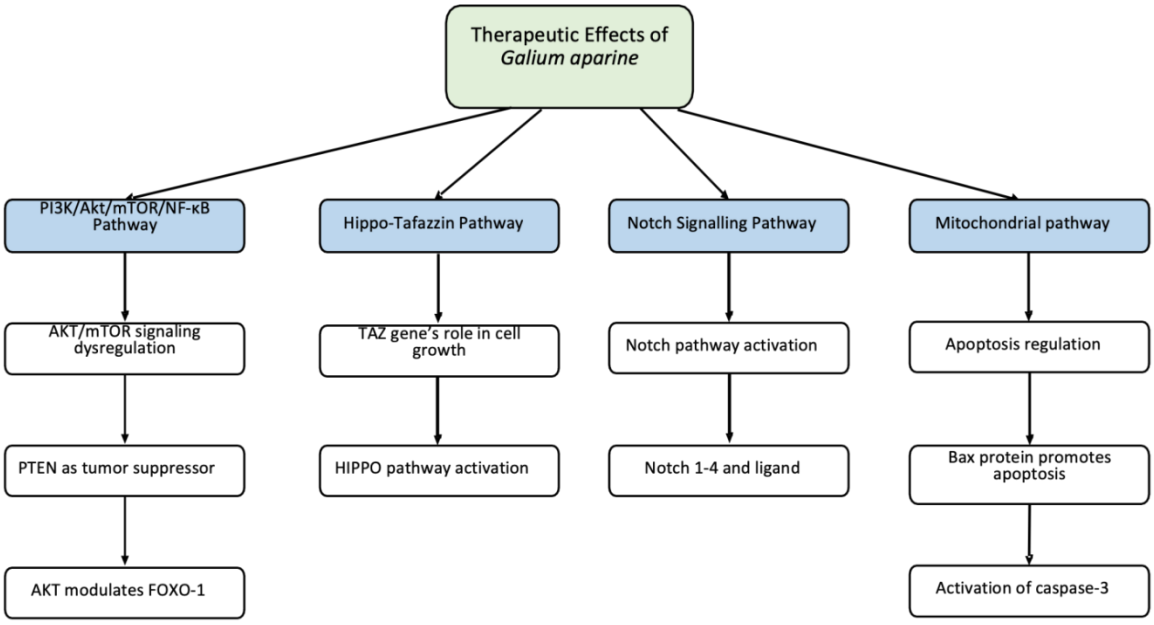

Figure 1.*Galium aparine* Biological Pathways [31], [32], [33], [34]

Key Signalling Pathways and their Disruptions in OSCC

The PI3K/Akt/mTOR/NF-κBSignalling Pathway

The OSCC initiation is associated with significant dysregulation of the Akt/mTOR pathway and persistent stimulation of NF-*κ*B. AKT/mTOR signalling pathways contribute significantly to numerous physiological phenomena, which include glycogen and fatty acid metabolism, autophagy, regulation of cell

cycle, protein synthesis, nutrient transporters and nuclear protein organisation. Additionally, it also affects the related signalling pathways like ERK, NF-κB, and JAK/STAT, affecting several cancer traits such as growth factors receptor-mediated mitogenic signals for cell proliferation survival signals for angiogenesis apoptosis invasion and migration [34].

Protein translation, apoptosis and tumour cell survival are processes regulated by the PI3K/AKT pathway. Activation of receptor tyrosine kinases stimulates PI3K to produce PIP3, which then phosphorylates AKT, which is part of the mTOR complex and promotes cancer cell's growth and survival on them. It has been observed that PTEN functions as a suppressor gene in tumours via reduced cyclin D1 expression, leading to G1 cell cycle arrest. AKT overexpression results from mutation-induced hyperactivity, hence reducing Bad or Bax apoptotic proteins for inhibiting apoptosis as it is pro-cell survival [35].

AKT also modulates FOXO-1, a transcription factor involved in the expression of proapoptotic proteins Bim and FasL, which generate apoptotic signals in DLO-RLSMC [36]. Interestingly, it is established that AKT's immediate focus downstream is the mTOR protein kinase, the activation of which stimulates cell division and tumour formation besides restraining cell death. The upstream GTPase Rab is activated through mTORC1 and increases the activity of the eIF4E complex to promote cell division and tumour proliferation. mTORC2 is involved in the complete phosphorylation of AKT at serine residues that are distinct from those responsible for phosphorylation by mTORC1. Interestingly, mTOR-dependent proteins have been reported to regulate the cell structure and growth through its downstream targets, mTORC1 and the newly described mTORC2.

The Hippo-Tafazzinsignalling Pathway

Tafazzin protein expressed by the TAZ gene has a crucial role in cell growth, survival, and cell movement by teaming up with transcription factors such as the TEAD family, which contributes to its involvement in tumour expansion. The Hippo pathway acts as a gatekeeper, preventing TAZ from entering the nucleus by adding phosphate groups to specific serine spots, thus keeping it within the cell's cytoplasm. TAZ's whereabouts are also affected by mechanical cues and signals from G protein-coupled receptors, both through pathways associated with Hippo and those that work independently. When the Hippo pathway gets activated, TAZ shifts into the nucleus, where it teams up with TEAD transcription factors and alters its gene expression further leading to rapid cell movement, growth and survival. In tongue squamous cell carcinoma (TSCC), when the Hippo-TAZ pathway is activated, it amps up cell growth, movement, and invasion while putting the brakes on apoptosis [27].

The Notch Signalling Pathway

A network of molecules Notch signalling pathway conveys signals across cells, which activates genes related to cell survival, angiogenesis and cell division. Increased presence in several types of cancer, including OSCC, leads to uncontrolled proliferation as well as metastasis [37]. Thus, when some molecules interact with the receptors located on the neighbouring cell surface, the Notch pathway is activated. In mammals, four sortilin cleavage δ -protocadherins (Notch 1, 2, 3, and 4) bind to the ligands- jagged (1 and 2) as well as delta-like (1, 2, and 4) and are involved in this signalling[38].

The Mitochondrial Pathway

Apoptosis, which is a form of cellular self-destruction, is pivotal in the proper functioning of immunity as it helps regulate their responses to various stimuli. Some proteins belonging to the Bcl-2 family contribute to the disruption of the outer mitochondrial membrane. Apoptosis, programmed cell death,

can be triggered in two main ways: two ways are (i) one that involves mitochondria (permeabilizing outer mitochondrial membrane) and (ii) another being through death receptors. The suggestion has been made that focussing on apoptosis could be an attractive approach for developing new anti-cancer therapies. In this pathway, Bax, a pro-apoptotic protein, promotes the release of cytochromes c into the cytosol. The final signal arises when cytochrome c associates with Apaf-1 to form an apoptosomal complex, which activates caspase-9 later. The activation process is finally converted into the activation of caspase-3 enzymes re- sponsible for the degradation of other proteins like PARP, hence promoting the execution of apoptosis. Caspase-3 executes depolymerization, which leads to apoptotic events at the cellular level [39].

Components from the cleaver extracts show potential in combating oral cancer. They do so by interacting with various pathways and genes, either activating or deactivating them. This interaction, whether direct or indirect, demonstrates their capability to slow down cancer cell growth or trigger apoptosis.

Advantages of Galium Aparine Over Standard Chemotherapeutic Agents

Chemotherapy, as a norm, is about the usage of anticancer drugs to stop the multiplication of cancer cells and/or to destroy those cells. These drugs are specifically created to fight off the rapidly dividing cells, however, they can be harmful to normal tissues which also multiply quickly, hence side effects may develop. The drugs are distributed all around the body through the bloodstream with this; they may do endobloc growth inhibitions, anti-vascular proliferations or direct killings of the cancerous cells. The medicines are available either intravenously (IV), orally or a combination of both [40].

Oral cancer chemotherapy often involves multiple medications. While combining drugs can enhance tumour reduction, it also increases the likelihood of side effects. Typically, these medications are administered sequentially and repeated every 2 to 3 weeks as part of a treatment cycle, which includes rest periods to allow healthy cells to recover. The frequency of the chemotherapy, which may be daily, weekly, or monthly depending on the patient's condition. Treatment duration generally spans from 2 to 6 months, based on its effectiveness. The common type of chemotherapy includes cisplatin, fluorouracil (5-FU), carboplatin, paclitaxel,and docetaxel [41].

These chemotherapy drugs are intended to target and destroy rapidly growing cancer cells. However, these drugs can also impact normal fast-growing cells, such as those in hair follicles, the intestines, the mouth and throat lining, and bone marrow, where blood cells are produced. The side effects of chemotherapy vary from person to person and typically subside once the treatment concludes. The most common side effects are anaemia, appetite loss, a change in the food taste, bloating, bruising or bleeding easily, constipation, dehydration, hair loss, susceptibility to risk of infection, nausea and vomiting [42].

The usage of *Galium aparine* in place of these chemo-compounds can be considered a better alternative. Being utilised as a traditional medicine it has multiple functions such as immunomodulator, cell death and restriction of cell proliferation and migration is evident in many. Therefore, multi-mechanistic medicines can be devised to combat cancer cells of different phenotypes. The efficacy of such drugs increases when more than one dys regulated pathway can be targeted at one shot making it better than extremely specific chemo- drugs that can regulate one to a few pathways. Also, the toxicity levels for the total flavonoids in Galium sp. may be safer to consume than chemo drugs [43].

Preclinical and Clinical Evidence

Preclinical Studies

Galium aparine is also a well-known folk medicine to treat jaundice and anxiety. The pre-clinical studies were previously done using *Galiumaparine-treated* rats that showed hepatoprotective potential. With the evolution of silver nanoparticles in the treatment of cancer, green synthesis using *G. aparine* is used in the treatment of skin cancer [44]. Similarly, breast cancer cells, when treated with the extract of cleavers, also showed a significant effect on cell viability and cell morbidity [45]. However, the studies on the treatment of OSCC by the species*G. aparine* are limited.

Overview of *In vitro* and *In vivo*Studies Demonstrating Efficacy against OSCC

In vitro trials with extracts of *G. aparine* are well documented in the treatment of various cancers. A study by Atmaca et al.,2016 conducted on three cell lines of breast cancer individually showed different anticancer effects (apoptotic, necrotic and cytotoxic) induced by 14 major phytochemicals and 34 volatile compounds in a concentration and time-dependent manner. In vitro study regarding methanol and ethyl acetate extract of *Galium aparine*'s aerial parts were tested for cytotoxic, apoptotic and antioxidant activity in colon and breast cancer cell lines and found the involvement of common phytochemicals like saponins, alkaloids, phenols, flavonoids (rutin) and others. The green biosynthesis of silver nanoparticles using aparian extract suggested that it is a good biological carrier with potential for pharmaceutical applications in skin cancers [43].

An in vivo study by Sahin et al., 2022 [46] suggests the action of *G. aparine* in acetaminophen-induced toxicity in mouse liver. A substantial reduction in necrosis, bilirubin and enzymes (aspartate/alanine aminotransferase) of the liver was noticed in the pretreated rats with unaffected hippocampal function and improved anxiolytic effect. This suggests that cleavers are potential substitutes for chemo-drugs.

Studies on oral cancers and HNC are done with a related Galium species (G. verum), and the aqueous extract significantly inhibits the migration of HNC cells and has protective actions against DNA damage in mucosal keratinocytes [37].

Analysis of Any Existing Clinical Trials, Outcomes, and Potential for Future Research

The components of the extract from G. verum are similar to that of *G. aparine*, which majorly includes iridoid flavonoids, thus suggesting aparian extracts that can be similarly used for the treatment of oral cancers. The protective actions against DNA damage caused by cigarette smoke are also shown to be replenished and are a safer option to acquire for mouth and upper digestive tract tumours[37]. Overall, it has considerable potential to be clinically tested and implied in biomedical industries. The previous reported clinical trials were reported in Table2.

Future Prospective Research

Galium concisely has extensive scientific and economic value for further uses in connection with the potential of the plant's extracts, such as apoptosis, necrosis, and cytotoxicity concerning cancer diseases. This potential stems from the chemical compounds that are found in it, which include flavonoids, iridoids and other bioactive ingredients. Various acidic and bitter components in *Galium aparine* include asperulosidic acid, asperuloside, acumine, monotropein, aucubin and other alkaloids such as protopine harmine. The plant also has other types of organic acids in its composition that belong to the class of hydroxycinnamic and phenolcarbonic acids, such as chlorogenic, caffeic, ferulic, and gentisic acids. It contains flavonoids that are

quercetin, rutin, hyperoside, kaempferol, astragalin and epicatechin, luteolin and apigenin, and phytosterols that embrace campesterol, stigmasterol and sitosterol [22].

The hydrolyzed aqueous extract of *Galium aparine* has been used for the treatment of numer- ous cancers, including breast, oral, and colon cancer, owing to its bioactive compounds like flavonoids, iridoids, saponins, and active volatile compounds [47]. Nonetheless, it is apparent that these compounds originating from natural sources like herbal extracts still have positive results that need further research and investigation. However, more in vivo experiments are necessary to explain the phenomena in question concerning the action of the compounds described above [22], [47].

Procedures that should be followed for future research include experiments on how yields from the plant can be maximised by using solvents with high potential for extraction of bioactive agents. This involves purifying and characterising these compounds and then posting further investigations on their uses in treating human malignant melanoma both in the inter and in vivo. Furthermore, the anticancer properties of the plant should be investigated further in terms of treatment of head and neck cancer cases. Performing such research would enhance the quality of life of patients suffering from the given diseases [22], [30].

Table 2. Previous Clinical Trials and Outcomes

Aspect	Findings	Reference
Cell lines tested	HLaC78 (larynx carcinoma)and FADU (hypopharyngeal carcinoma) cell lines were utilized in the study	[48]
Toxicity on cell lines	The aqueous extractinhibited HLaC78 and FADU cell growth at high doses. MDR-1-expressing FADU cells were less sensitive. Primary mucosal keratinocytes, withoutp-glycoprotein, were minimally affected even athigh Galium concentrations.	[48]
Motility Inhibition	Strong inhibition of motilityin HLaC78 and FADU spheroidal cells on Matrigel-coated surfaces	[48][16]
DNA Protection	Galium decoction protectedDNA of primary mucosal keratinocytes against benzo[a]pyrene damage	[48]
Experimental Methods	Techniques includedspheroid motility assays, qRT-PCR, gelatin zymography, and MTT cytotoxicity tests.	[48]

Discussion and Conclusion

Globally, head-and-neck squamous cell carcinomas (HNSCC) rank as the 18th most prevalent cancer type. Ninety percent of HNSCCs are oral squamous cell carcinomas (OSCCs), the most common kind that develops in the oral cavity. The grade, stage, metastasis, and invasiveness of the malignancy are among the prognostic factors that affect the survival rate of malignancies. There is a continuous search for new biomarkers and treatment targets because of the complex pathophysiology of cancer and the low survival rates in advanced illnesses.[49].

Additionally, these methods have a lot of drawbacks and serious side effects, which has prompted researchers to look for more effective therapies. This paper reveals that one of the most significant sources of relief for OSCC— *Galium aparine*, a medicinal plant, can be of much help in combating the hazardous. This plant also contains different bioactive compounds like flavonoids, iridoids, and phenolic acids, which have antioxidant anticancer immunomodulatory potential [5].

Based on previous investigations, particularly on animal models, *G. aparine* has been demonstrated to possess significant cancer-fighting ability against various types of carcinomas, including the breast and skin [9]. As a molecule with a modulatory impact on some of the most cardinal signalling pathways linked with OSCC, including the PI3K/Akt/mTOR, Hippo-TAZ, and Notch pathways, it can augment its therapeutic potential for OSCC treatment. Besides, *G. aparine* can also boost the immune response, decrease the hazardous effects caused by conventional chemotherapeutic agents, and have minimal side effects [25,50].

Therefore, the therapeutic use of *G. aparine* in OSCC is still potential since the herb contains diverse biological activities [8]. This is because of its phytochemical contents, including flavonoids and iridoids, which have health benefits such as antioxidants and/or anticancer properties. These compounds can, therefore, prevent further multiplication of cancer cells, promote their destruction, and control the immune system, making *G. aparine* a potentially useful complement or substitute for standard chemotherapy [10], [25].

The PI3K/Akt/mTOR is one of the most significant signalling cascades that stimulates cell survival and growth in the context of cancer. Written as such, *G. aparine* interferes with this pathway, in addition to the Hippo-TAZ and the Notch pathways, suggesting the molecule's capability to target OSCC accurately. Moreover, the pro-apoptotic role of the plant in the mitochondria pathway serves as the basis for further investigations on the anticancer potentials of the plant [36].

The advantage of using *G. aparine* over standard chemotherapy is that it does not pose such a high risk of toxicity. With GA having high biological activity and the potential to be used as a natural pharmacological agent, a research study by Atmaca et al. suggests that GA MeOH extract may have potential anti-cancer effects against breast cancer cells without impairing normal breast epithelial cells, making it a better alternative to traditional chemotherapy drugs [45]. Chemotherapy comes with several setbacks, such as myelosuppression, nausea, and multidrug resistance, which hinder its functioning. While the synthetic compounds of *G. aparine* have side effect profiles that appear more severe, naturally occurring compounds of the plant are less injurious and, hence, safer for use in long-term treatment.

The current work emphasises basic research exploring the therapeutic properties of *G. aparine* or its components, but clinical trials have proved pivotal in translating scientific knowledge into practice. Although there is great potential for healing using natural resources, their inherent qualities need to be thoroughly examined to make sure they are safe for human use [51]. Mineralisation of artificial substitutes like calcium carbonate, prf, nano hydroxyapatite have shown to have clinical benefits across various fields [52].

To assist the shift from experimental research to clinical applications, more funded studies with bigger sample sizes are required in the future to investigate the anti-cancer potential of Galium aparine in OSCC. Even if research conducted in vivo and in vitro has produced encouraging results, clinical trials are necessary to advance the effects of *Galium aparine* in the treatment of cancer. Further studies should be carried out with well-controlled, double-blind, and randomised

clinical trials that compare the effectiveness and toxicity of *G. aparine* in OSCC patients [47]. Furthermore, the identification of the possible synergistic activities of *G. aparine* and other chemo drugs may help to identify new drug synergisms, leading to more effective treatment options that also reduce the adverse effects of chemotreatment.

In conclusion, the information derived from the present study provided strong evidence supporting further investigation and development of the use of *Galium aparine* as a therapeutic option for OSCC. Being endowed with several phytochemicals and given the findings of this study on its potential to modulate OSCC cancer and improve the immune response mechanism, this plant could greatly help enhance the survival and well-being of OSCC patients.

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Conflict of Interest

Nil.

References

[1]. Meier, J. K., Schuderer, J. G., Zeman, F., Klingelhöffer, C., Hullmann, M., Spanier, G., & Ettl, T., 2019, Health-related quality of life: a retrospective study on local vs. microvascular reconstruction in patients with oral cancer. BMC oral health, 19, 1-8.

[2]. Bagan, J., Sarrion, G. and Jimenez, Y., 2010, Oral cancer: clinical features. Oral oncology, 46(6), pp.414-417.

[3]. Warnakulasuriya, S., 2020, Oral potentially malignant disorders: A comprehensive review on clinical aspects and management. Oral Oncology, 102, p.104550.

[4]. Tarakji, B., 2022, Dentists' perception of oral potentially malignant disorders. International Dental Journal, 72(3), pp.414-419.

[5]. Kerr, A.R. and Lodi, G., 2021, Management of oral potentially malignant disorders. Oral Diseases, 27(8), pp.2008-2025.

[6]. Selvaraj FM, Pillai VR, Joseph AP, Ramani P, Pazhani J, Mony V. Assessment of Tumor Budding in Different Grades of Oral Squamous Cell Carcinoma. Journal of Orofacial Sciences. 2023 Jul 1;15(2):160-6.

[7]. Tuorkey, M.J., 2015, Cancer therapy with phytochemicals: present and future perspectives. Biomedical and Environmental Sciences, 28(11), pp.808-819.

[8]. Orhan, N., Orhan, D.D., Aslan, M., Şüküroğlu, M. and Orhan, I.E., 2012, UPLC–TOF-MS analysis of Galium spurium towards its neuroprotective and anticonvulsant activities. Journal of ethnopharmacology, 141(1), pp.220-227.

[9]. Bokhari, J., Khan, M.R., Shabbir, M., Rashid, U., Jan, S. and Zai, J.A., 2013, Evaluation of diverse antioxidant activities of Galium aparine. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 102, pp.24-29.

[10]. Deliorman, D.İ.D.E.M., Calis, I. and Ergun, F., 2001, Iridoids from Galium aparine. Pharmaceutical Biology, 39(3), pp.234-235.

[11]. Simon, D., 2018, Recent advances in clinical allergy and immunology. International archives of allergy and immunology, 177(4), pp.324-333.

[12]. Vorob'ev, A.A., 2002, Principles of classification and the strategy of immunomodulators used in medicine. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii, (4), pp.93-98.

[13]. Vidalain, J.P., Onimus, M. and Michel, C.R., 1975, Long-term results of sub-astragal and mediotarsal arthrodesis. Revue de chirurgie orthopedique et reparatrice de l'appareil moteur, 61, pp.301-306.

[14]. Sun, H. X., Xie, Y. and Ye, Y. P., 2009, Advances in saponin-based adjuvants. Vaccine, 27(12), pp.1787-1796.

[15]. Mocan, A., Crișan, G., Vlase, L., Crișan, O., Vodnar, D.C., Raita, O., Gheldiu, A.M., Toiu, A.,

Oprean, R. and Tilea, I., 2014, Comparative studies on polyphenolic composition, antioxidant and antimicrobial activities of Schisandra chinensis leaves and fruits. Molecules, 19(9), pp.15162-15179.

[16]. Taylor, K., 1999, Galium aparine L. Journal of Ecology, 87(4), pp.713-730.

[17]. Mitova, M.I., Anchev, M.E., Handjieva, N.V. and Popov, S.S., 2002, Iridoid patterns in Galium L. and some phylogenetic considerations. Zeitschrift für Naturforschung C, 57(3-4), pp.226-234.

[18]. Ilina, T., Kashpur, N., Granica, S., Bazylko, A., Shinkovenko, I., Kovalyova, A., Goryacha, O. and Koshovyi, O., 2019, Phytochemical profiles and in vitro immunomodulatory activity of ethanolic extracts from Galium aparine L. Plants, 8(12), p.541.

[19]. Al-Snafi, A.E., 2022, Constituents, nutritional and pharmacological importance of Prunus persica-A review. World Journal of Advanced Pharmaceutical and Medical Research, 3(1), pp.019-029.

[20]. Kanso, M.A., Hijazi, M.A., El-Lakany, A. and Aboul-Ela, M., 2024, Review on phytochemical constituents and pharmacological activities of genus Galium. Journal of Applied Pharmaceutical Science.

[21]. Kuhtinskaja, M., Bragina, O., Kulp, M. and Vaher, M., 2020, Anticancer effect of the iridoid glycoside fraction from Dipsacus fullonum L. leaves. Natural Product Communications, 15(9), p.1934578X20951417.

[22]. Saar-Reismaa, P., Koel, M., Tarto, R. and Vaher, M., 2022, Extraction of bioactive compounds from *Dipsacus fullonum*leave using deep eutectic solvents. Journal of Chromatography A, 1677, p.463330.

[23]. Vorob'ev, A.A., 2002, Principles of classification and the strategy of immunomodulators used in medicine. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii, (4), pp.93-98.

[24]. Dai, J. and Mumper, R.J., 2010, Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15(10), pp.7313-7352.

[25]. Ilina, T., Skowrońska, W., Kashpur, N., Granica, S., Bazylko, A., Kovalyova, A., Goryacha, O. and Koshovyi, O., 2020, Immunomodulatory activity and phytochemical profile of infusions from Cleavers herb. Molecules, 25(16), p.3721.

[26]. Bradic, J., Petkovic, A. and Tomovic, M., 2021, Phytochemical and Pharmacological properties of some species of the genus. Experimental and Applied Biomedical Research (EABR), 22(3), pp.187-193.

[27]. Arts, I.C. and Hollman, P.C., 2005, Polyphenols and disease risk in epidemiologic studies. The American journal of clinical nutrition, 81(1), pp.317S-325S.

[28]. Manach, C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L., 2004, Polyphenols: food sources and bioavailability. The American journal of clinical nutrition, 79(5), pp.727-747.

[29]. Wang, C., Gong, X., Bo, A., Zhang, L., Zhang, M., Zang, E., Zhang, C. and Li, M., 2020, Iridoids: research advances in their phytochemistry, biological activities, and pharmacokinetics. Molecules, 25(2), p.287.

[30]. Liu, S., Khan, A.R., Yang, X., Dong, B., Ji, J. and Zhai, G., 2021, The reversal of chemotherapy-induced multidrug resistance by nanomedicine for cancer therapy. Journal of Controlled Release, 335, pp.1-20.

[31]. Naeem, A., Hu, P., Yang, M., Zhang, J., Liu, Y., Zhu, W. and Zheng, Q., 2022, Natural products as anticancer agents: current status and future perspectives. Molecules, 27(23), p.8367.

[32]. Harsha, C., Banik, K., Ang, H.L., Girisa, S., Vikkurthi, R., Parama, D., Rana, V., Shabnam, B., Khatoon, E., Kumar, A.P. and Kunnumakkara, A.B., 2020, Targeting AKT/mTOR in oral cancer: mechanisms and advances in clinical trials. International journal of molecular sciences, 21(9), p.3285.

[33]. Christianto, S., Li, K.Y., Huang, T.H. and Su, Y.X., 2022, The Prognostic Value of Human Papilloma Virus Infection in Oral Cavity Squamous Cell Carcinoma: A Meta-Analysis. The Laryngoscope, 132(9), pp.1760-1770.

[34]. Cavalcante, G.C., Schaan, A.P., Cabral, G.F., Santana-da-Silva, M.N., Pinto, P., Vidal, A.F. and Ribeiro-dos-Santos, Â., 2019, A cell's fate: an overview of the molecular biology and genetics of apoptosis. International journal of molecular sciences, 20(17), p.4133.

[35]. Schmidt, M., Polednik, C., Roller, J. and Hagen, R., 2014, Galium verum aqueous extract strongly inhibits the motility of head and neck cancer cell lines and protects mucosal keratinocytes against toxic DNA damage. Oncology Reports, 32(3), pp.1296-1302.

[36]. Zhang, J., Zheng, G., Zhou, L., Li, P., Yun, M., Shi, Q., Wang, T. and Wu, X., 2018, Notch signalling induces epithelial-mesenchymal transition to promote metastasis in oral squamous cell carcinoma. International journal of molecular medicine, 42(4), pp.2276-2284.

[37]. Zong, W.X., Rabinowitz, J.D. and White, E., 2016, Mitochondria and cancer. Molecular cell, 61(5), pp.667-676.

[38]. Carr, C., Ng, J. and Wigmore, T., 2008, The side effects of chemotherapeutic agents. Current Anaesthesia & Critical Care, 19(2), pp.70-79.

[39]. Kelland, L., 2007, The resurgence of platinum-based cancer chemotherapy. Nature Reviews Cancer, 7(8), pp.573-584.

[40]. Kayl, A.E. and Meyers, C.A., 2006, Side-effects of chemotherapy and quality of life in ovarian and breast cancer patients. Current opinion in obstetrics and gynaecology, 18(1), pp.24-28.

[41]. Laanet, P.R., Saar-Reismaa, P., Jõul, P., Bragina, O. and Vaher, M., 2023, Phytochemical screening and antioxidant activity of selected Estonian Galium species. Molecules, 28(6), p.2867.

[42]. Hamdi, O.H., Saadedin, S.M. and Al_Zaidi, I.H., 2021, Green biosynthesis of silver nanoparticles using gallium aparine green part extract and anti-skin cancer activity. Medico-legal Update, 21(2), pp.908-913.

[43]. Atmaca, H., Bozkurt, E., Cittan, M. and Tepe, H.D., 2016, Effects of Galium aparine extract on the cell viability, cell cycle and cell death in breast cancer cell lines. Journal of ethnopharmacology, 186, pp.305-310.

[44]. Sahin, B., Karabulut, S., Filiz, A.K., Özkaraca, M., Gezer, A., Akpulat, H.A. and Ataseven, H., 2022, Galium aparine L. protects against acetaminophen-induced hepatotoxicity in rats. Chemico-Biological Interactions, 366, p.110119.

[45]. Mazzio, E.A. and Soliman, K.F., 2010, In vitro screening of tumoricidal properties of international medicinal herbs: part II. Phytotherapy Research, 24(12), pp.1813-1824.

[46]. Corrigan, D., Timoney, R.F. and Donnelly, D.M., 1978, Iridoids and alkanes in twelve species of Galium and Asperula. Phytochemistry, 17(7), pp.1131-1133.

[47]. Ramasubramanian A, Arumugam P, Ramani P, Kannan BC, Murugan MS. Identification of Novel Cytochrome C1 (CYC1) Gene Expression in Oral Squamous Cell Carcinoma- An Evaluative Study. Ann Maxillofac Surg. 2022 Jul-Dec;12(2):144-150. doi: 10.4103/ams.ams_26_22. Epub 2022 Aug 24. PMID: 36874769; PMCID: PMC9976869.

[48]. Renu, K., 2024. A molecular viewpoint of the intricate relationships among HNSCC, HPV infections, and the oral microbiota dysbiosis. *Journal of Stomatology, Oral and Maxillofacial Surgery*, p.102134.

[49]. Subramanian, A.K. and Balakrishnan, N., Evaluation of Biological Response Elicited by Two Novel Tooth Cream Formulations of Cocos nucifera-Cell Line Studies and MTT Assay on Human Gingival Fibroblast.

[50]. Kaarthikeyan, G., Jayakumar, N.D. and Sivakumar, D., 2019. Comparative Evaluation of Bone Formation between PRF and Blood Clot Alone as the Sole Sinus-Filling Material in Maxillary Sinus Augmentation with the Implant as a Tent Pole: A Randomized Split-Mouth Study. *Journal of long-term effects of medical implants*, *29*(2).

[51]. Kavarthapu, A. and Malaiappan, S., 2019. Comparative evaluation of demineralized bone matrix and type II collagen membrane versus eggshell powder as a graft material and membrane in rat model. *Indian Journal of Dental Research*, *30*(6), pp.877-880.

[52]. Manchery, N., John, J., Nagappan, N.,

Subbiah, G.K. and Premnath, P., 2019. Remineralization potential of dentifrice containing nanohydroxyapatite on artificial carious lesions of enamel: A comparative: in vitro: study. *Dental research journal*, *16*(5), pp.310-317.