"EVALUATION AND COMPARISON OF EFFICACY OF POLYHERBAL GEL CONTAINING ASHWAGANDHA AND GINGER AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS – AN IN-VIVO STUDY"

By
REG. NO. IK0219004

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REG. NO. IK0219004

Dr. Vinayak Kumbhojkar
Consultant
Periodontics, KLE V.K IDS, Belgaum

Dr. VINAYAK KUMBHOJKAR *M.D.S*
Professor and Head,
Department of Periodontics, KAHER's
KLE V. K. Instituteof Dental Sciences,
Belagavi.

Professor and Head
Department of Periodontics
KLE V. K. Institute of Dental Sciences
Belagavi

Date: 06/01/2022
Place: Belagavi

Dr. ALKA KALE *M.D.S, Ph.D.*
Principal,
KAHER's KLE V. K. Institute
of Dental Sciences,
Belagavi.

PRINCIPAL
KLE V. K. Institute of Dental Sciences
Nehru Nagar, Belagavi

Date: 6/1/22
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Nehru Nagar, Belagavi - 590 010, Karnataka State

Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Date : 5-1-2022 Serial No. : 104

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UG / PG / Ph.D / Staff : POST GRADUATE
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Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)
: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

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ABSTRACT

Background:

Periodontitis is a multifactorial, infectious chronic inflammatory disease of periodontium having the ability to initiate immune response causing periodontal destruction eventually leading to bone loss. The primary goal of periodontal therapy is to reduce microbial load using various non-surgical approaches mainly through scaling and root planing which is considered as a gold standard. It fails to eliminate the pathogenic bacteria completely especially at the base of the periodontal pocket and the areas inaccessible to periodontal instruments. Therefore, the use of local drug delivery in the affected area using synthetic antimicrobials was introduced as an adjunct to scaling and root planing. To overcome the side-effects of these synthetic drugs, plant-based medicinal substitutes were incorporated alone or in combination in the treatment of chronic periodontitis.

Aim:

To evaluate and compare the efficacy of a polyherbal gel containing Ashwagandha and Ginger as a Local Drug Delivery Agent as an adjunct to scaling and root planing, on the clinical and biochemical parameters in patients with chronic periodontitis.

Materials and methods:

A total of 23 patients with chronic periodontitis following eligibility criteria who reported to KAHER's KLE Vishwanath Katti Institute of Dental Sciences, Belagavi were considered for the study. The study was a randomized, controlled, double-blinded clinical trial with a split-mouth design. For each patient, two sites in contralateral quadrants were selected. The sites were randomly divided using the coin-

toss method into test and control groups. In the control sites (n=23), scaling and root planing was performed, whereas in the test sites (n=23) local drug delivery of polyherbal gel containing Ashwagandha and Ginger was used as an adjunct to Scaling and root planing. (SRP + polyherbal gel). The clinical parameters recorded at baseline and at 4 weeks post-treatment from each site were Plaque Index (PI), Sulcus Bleeding Index (SBI), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL). The biochemical parameter accessed from gingival crevicular fluid was levels of Alkaline phosphatase (GCF ALP). The statistical analysis was performed using SPSS Software 20.00. Intra-group comparison from baseline to 4 weeks for both the test and control groups including clinical and biochemical parameters was done using paired t-test, while for intergroup comparison between test and control groups Z-test was used. The p-value <0.05 was considered as statistically significant and $p<0.001$ as statistically highly significant.

Results:

Intra-group comparison from baseline to 4 weeks post-treatment in both test and control groups showed statistically significant ($p<0.001$) improvement in all the clinical and biochemical parameters. However, an inter-group comparison between the test and control group showed statistically significant improvement (reduction in PI, SBI, PPD, CAL and GCF ALP) in the test group than control group ($p<0.001$). This could be due to anti-inflammatory, anti-bacterial, wound healing and anti-oxidant properties of both Ashwagandha and ginger present in the polyherbal gel.

Conclusion:

In the current study, the administration of Polyherbal gel containing Ashwagandha and Ginger as a local drug delivery agent as an adjunct to scaling and root planing has

a synergistic effect to scaling and root planning in the treatment of chronic periodontitis. This was ascribed to immunomodulatory, anti-inflammatory, anti-microbial and anti-oxidant properties of Ashwagandha as well as analgesic, anti-inflammatory, antioxidant and anti-bacterial actions of Ginger combined in the form of a polyherbal gel.

Keywords: Chronic Periodontitis, Scaling and Root Planing, Local Drug Delivery, Gingival Crevicular Fluid, Polyherbal gel, Ashwagandha, Ginger, Alkaline Phosphatase

LIST OF ABBREVIATIONS

SRP	Scaling and Root planing
LDD	Local Drug Delivery Agent
PI	Plaque Index
SBI	Sulcus Bleeding Index
PPD	Probing Pocket Depth
CAL	Clinical Attachment Level
GCF	Gingival Crevicular Fluid
ALP	Alkaline phosphatase levels
GCFALP	Alkaline phosphatase levels in Gingival Crevicular Fluid
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
PMNs	Polymorphonuclear neutrophils
ROS	Reactive oxygen species
MMPs	Matrix Metalloproteinases
WS	Ashwagandha, *Withania somnifera* (L.) Dunal
IL-1	Interleukin-1
IL-6	Interleukin-6
TNF	Tumor necrosis factor
IFN	Interferon
WA	Withaferin A

PG/ PGE2	Prostaglandin/ Prostaglandin-E2
COX	Cyclo-oxygenase
LOX	Lypo-oxygenase
Na CMC	Sodium Carboxymethylcellulose
MGI	Modified Gingival Index
GBI	Gingival Bleeding Index
MQH	modified Quigley and Hein index
GM-CSF	Granulocyte/Macrophage-Colony Stimulating Factor
NSAID	Non-steroidal anti-inflammatory drugs
NMR	Nuclear Magnetic Resonance
HPLC	High-performance Liquid Chromatography
GC MS	Gas Chromatography-Mass Spectrometry
WS-AgNPs	Ashwagandha-Silver Nanoparticles
CFUs	Colony Forming Units
m-RNA	Messenger- Ribonucleic acid
BA	Botanical Bio-actives
TAR	Targets
NF-κB	Nuclear factor-kappa B
NEP	Network ethnopharmacology
Hb	Hemoglobin
RBC	Red-blood cells

NEMO/IKKβ	NF-κB Essential Modulator/ IκB kinase
ProCT	Procalcitonin
SSI	Stainless steel implant
RT-PCR	Reverse transcriptase-polymerase chain reaction
TLC	Thin-layer chromatography
SOD	Superoxide Dismutase
CAT	Catalase
GPX	General Peroxidases
PI*	Proteasomal Inhibitor
RANK	Receptor Activator of Nuclear Factor kappa-B
BMP	Bone morphogenic protein
CAM	Complementary and Alternative Medicine
A.a	*Aggregatibacter actinomycetemcomitans*
EPS	Microbial Epoxypolysaccharides
VAS	Visual Analog scale
NSPT	Non-surgical periodontal therapy
iNOS	Nitric Oxide synthase
NGP	Neutral ginger polysaccharide
NO	Nitric oxide
NMR	Nuclear Magnetic Resonance
LPO	Lipid Peroxidation

HAPC	Hydroxymethoxyphenyl compound
MCP	monocyte chemotactic protein
THP-1	human leukemia monocytic cell line
RAL	relative attachment level
AAP	American academy of periodontology
KAHER	KLE Academy of Higher Education and Research
BHI	Brain heart infusion
DMSO	Dimethyl sulfoxide
PEG 400	Polyethylene glycol 400
p-value	Probability value
SPSS	Statistical package for the social sciences
CHX	chlorhexidine
DM	Diabetes mellitus
QS	Quorum sensing

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INTRODUCTION

Periodontitis is a multifactorial disease involving inflammation of the tissues surrounding teeth initiated by bacteria.[1] Though the infection with specific periodontal pathogenic bacteria is necessary for periodontitis to develop, it alone is insufficient for the development of the disease.[2] Therefore, periodontitis is considered as the net effect of the immune response and the inflammatory processes.[3] If there is a further increase in periodontopathogens in the oral cavity, it will lead to stimulated immune response leading to a drastic increase in the levels of polymorphonuclear neutrophils (PMNs). This will eventually initiate the production of reactive oxygen species (ROS), thereby allowing the differentiation of osteoclasts, inflammatory modulators and production of several proteolytic enzymes like matrix metalloproteinases (MMPs) causing an increase in inflammation furthermore. Additionally, it has been evidenced that increased oxidative stress and diminished antioxidant capacity are major factors accountable for the destruction of periodontal structures. Altogether, this will ultimately lead to the destruction of the surrounding periodontium aggravating periodontitis.[2]

The primary objective of periodontal therapy is to reduce the microbial load, using various non-surgical and surgical approaches. To date, scaling and root planing (SRP) is the most widely used non-surgical approach.[4] Nevertheless, complete mechanical debridement is difficult to achieve especially in sites with deep periodontal pockets and in the areas inaccessible to periodontal instrumentation, the chief reason being the presence of tissue invasive pathogenic microflora within gingival tissues and root surface complexities.[5] With emerging awareness concerning the role of oxidative stress associated with periodontal disease, host-modulating

therapies are being investigated which regulate the role of antioxidants in the prevention of the breakdown of soft and hard periodontal tissues.[6] It has been proved that, even if adjunctive administration of local antibiotics and chemotherapeutic agents is essential for the better periodontal outcome, it may result in higher systemic doses of the concerned drug, development of the bacterial resistance and disruption of commensal microflora.[5]

Therefore, Goodson in 1979 first proposed the concept of controlled delivery in the treatment of periodontitis which attains up to 100-fold higher concentrations of an antimicrobial agent in subgingival sites than a systemic drug regimen.[4,7] In the arena of local drug delivery (LDD), to overcome these side effects of synthetic molecules like tetracycline, doxycycline, metronidazole and chlorhexidine gluconate,[4] the effective use of medicinal plants like curcumin, green tea, aloe-vera, pomegranate and neem was suggested as an adjunct to scaling and root planning in the literature.[8]

To enhance the potential of these herbal drugs, even more, they can be used in combinations to alleviate their synergistic action. This is termed as "Herbal shotgun" or "synergistic multitarget effects".[9] In the current study, we incorporated Ashwagandha and Ginger together in the form of polyherbal gel to determine their synergistic action both in-vitro and in-vivo.

Ashwagandha (Botanical name – *Withania somnifera*) is one of the important medicinal herbs of Ayurveda.[10] It belongs to the *Solanaceae* family and possesses medicinal value in its leaves, roots and fruits.[11] The main constituents of ashwagandha are Withaferin A (WA), a biologically active steroid in the leaf extract along with alkaloids and steroidal lactones.[12] It has been found that Ashwagandha is a potential antimicrobial agent with antifungal activity and moderate antibacterial activity against

Staphylococcus aureus and *Pseudomonas aeruginosa*.[11] It also possesses antioxidant, anti-inflammatory, anti-cancer and immunoregulatory properties which can further modulate cytokines such as IL-1, IL-6, TNF and IFN.[12] Results of various studies suggested that withaferin A also inhibits Prostaglandin-E2 (PGE2) production and cyclo-oxygenase-2 (COX-2) expression demonstrating its non-selective nature.[13]

To our knowledge, to date, there are no reports of the use of Ashwagandha as an LDD agent in the treatment of periodontal diseases.

Ginger (Botanical name – *Zingiber officinale Roscoe*) is a creeping perennial underground rhizome of the family – *Zingiberaceae*, known as "Sunthi in Ayurveda". It has a medicinal part as a dried rhizome with characteristic pungent and aromatic odor and taste.[14] All major active ingredients of ginger, including gingerols, zingerone, gingerdiol, zingiberene and shogaols are known to possess antioxidant activities as it diminishes or prevents generation of free radicals.[14] Kiuchi et al. were the first to display that those extracts of plants belonging to the *Zingiberaceae* family, inhibit PG synthesis in vitro. A study by Tjendraputra et al. showed that gingerols being more potent inhibitors of COX-1 than COX-2 is recognized as a non-selective COX inhibitor showing broad-spectrum anti-inflammatory activity of ginger.[15] It showed antibacterial activities as well against anaerobic gram-negative periodontopathogens like *Porphyromonas gingivalis* and *Prevotella intermedia*. There are reports of Ginger inhibiting biofilm formation and even altering its phenotype.[16]

In the assessment of progress and efficacy of the treatment of periodontitis, diagnostic markers play a pivotal role. The ecology of the periodontal pocket is determined by GCF as it seeps into gingival crevices or periodontal pockets around teeth with inflamed gingiva as an inflammatory exudate.[17,18] The analysis of

biomarkers in Gingival Crevicular Fluid (GCF) might be considered helpful in forecasting patient vulnerability to future attachment loss and it also can have the possibility of describing the inflammatory events occurring at the particular site.[19] GCF has the advantage over other biomarkers as its collection is noninvasive, site-specific about teeth, comparatively easy to perform and offers one of the most accessible entries of any tissue in the body as a means of assessing the disease state.[17]

Alkaline phosphatase (ALP) is one of the first diagnostic markers identified in GCF which was first described in the literature by Suzuki U Yoshimura and Takaisi K in 1907. Alkaline Phosphatase belongs to the class hydrolases and the subclass Esterase. It is an Orthophosphoric monoester polyhydrolase being a nonspecific phosphate.[20] The distribution of ALP in periodontal tissues in gingivitis and periodontitis was studied by Carranza and Cabrini where they demonstrated that ALP was found in large amounts in the granulation tissue of the lateral wall and at the bottom of the periodontal pocket along with the gingival fibres at the point of their insertion.[18] The ratio of GCF to serum alkaline phosphatase was found to be ranging from 6:1 to 11:1, acclaiming its origin majorly from its local production. It is stated, that the increase in alkaline phosphatase in periodontal disease indicate a metabolic reaction of the enzyme against the tissue changes caused by inflammation and the concentration of Alkaline phosphatase was found to be positively associated with periodontal disease activity.[18]

Thus, the present study was designed with an aim of evaluating and comparing the efficacy of polyherbal gel containing Ashwagandha and Ginger as an adjunct to scaling and root planning in the treatment of chronic periodontitis with clinical and biochemical parameters.

AIMS AND OBJECTIVES

AIM OF THE STUDY: -

To evaluate and compare the efficacy of a polyherbal gel containing Ashwagandha and Ginger as a Local Drug Delivery Agent as an adjunct to scaling and root planing, on the clinical and biochemical parameters in patients with chronic periodontitis.

OBJECTIVES OF THE STUDY: -

- To evaluate the efficacy of scaling and root planing on clinical parameters and alkaline phosphatase levels in gingival crevicular fluid (GCF) in patients with chronic periodontitis.
- To evaluate the efficacy of locally delivered polyherbal gel containing Ashwagandha and Ginger as an adjunct to scaling and root planing on clinical parameters and alkaline phosphatase levels in gingival crevicular fluid (GCF) in patients with chronic periodontitis.
- To compare the efficacy of locally delivered polyherbal gel containing Ashwagandha and Ginger as an adjunct to scaling and root planing and scaling and root planing alone on clinical parameters and alkaline phosphatase levels in gingival crevicular fluid (GCF) in patients with chronic periodontitis.

REVIEW OF LITERATURE

Periodontal disease is a complex, multifactorial, chronic inflammatory disease, affecting the periodontal tissue with the initiation of the host inflammatory reaction primarily due to the presence of local factors like periodontopathogens leading to further destruction if not controlled at the initial stage. Therefore, the primary goal of periodontal therapy remains as reducing microbial load using various non-surgical and surgical periodontal therapies, out of which, scaling and root planing still remain as a gold standard.

Literature suggests, use of several chemotherapeutic agents like tetracycline, doxycycline, metronidazole either systemically or locally to achieve complete elimination of disease-causing pathogens, as an adjunct to mechanical instrumentation. Pulling the same thread, Goodson in 1979 first proposed the concept of controlled delivery in the treatment of periodontitis[7], where he observed that local drug delivery can attain up to 100-fold higher concentrations of desired antimicrobial agents in subgingival sites than that of a systemic drug regimen.[4] To avoid side effects caused by synthetic drugs, one of the many alternatives such as the use of plant-based medicinal formulations was considered which is the current topic for research mainly due to its lower cost, easy availability and fewer side effects. To increase their medicinal value furthermore, these herbs are being used in combinations to help achieve their synergistic actions.

Polyherbal formulations

In a study by Alawdi SH et al. conducted in 2019, the author formulated herbal gel containing alcoholic extracts of *Withania somnifera* (Ashwagandha), *Allium sativum* (Garlic), and *Curcuma longa* (Turmeric) and investigated its wound

healing activities in thirty adult male Rabbits after topical application on full-thickness skin wounds (induced surgically). Animals were randomly allocated into 6 groups each containing 6 animals. Group 1 – without treatment (control). Group 2 – the carbomer gel base without drugs. Group 3 – herbal gel containing Ashwagandha extract. Group 4 – herbal gel containing Garlic extract. Group 5 – herbal gel containing Turmeric extract and Group 6 – gel containing Ciprofloxacin. The gel was applied on the wound once a day up to 14-18 days starting from the 2^{nd} day of occurrence of the wound. Whereas, in the animals treated with Ashwagandha gel, it was observed that wound healing reached about 90% by the end of the 12^{th} day and completely healed by the 14^{th} day. While, in Garlic gel-treated rats, it reached about 90% by the end of the 10^{th} day and reached up to 97% by the 12^{th} day followed by complete healing by the 14^{th} day.[21]

Aslani A et al. (2018) in their study proved that Gels are the better options for their higher aqueous content, lower dermal irritations and lesser mechanical abrasion with more esthetic appearance for its topical use on and around lips when compared to other topical drugs. Here, 2.5% hydro-alcoholic extracts of Melissa, Sumac, Rosemary and licorice were added in equal quantity to hydrophilic gel with 0.002% of Geranium essence. In total, 14 formulations were prepared with the use of gelling polymers such as Carbopol 940, Sodium carboxymethylcellulose and Hydroxypropyl methylcellulose, Carbopol and sodium carboxymethylcellulose (Na CMC). It was observed that, with an increase in the amount of polymer, the gel became thicker along with limited water penetration leading to a reduction in drug release. In the case of Carbopol – its increase raised mucoadhesion, viscosity and decreased drug release rate. Therefore, Carbopol plays a major role in the release of drugs from gel because of its high molecular weight and degree of crosslinking. In this study, rosemary and

licorice were thought to be responsible for anti-inflammatory, analgesic, repairing and wound healing effects. As the results of evaluation tests (mucoadhesion, viscosity and drug release profile) suggested, a formulation containing 3% Na CMC and 1% Carbopol 940, was selected for its acceptable viscosity and mucoadhesion. Its drug release profile Study indicated that herbal extracts can be released over a 24-hour period.[22]

Mahyari S et al. (2016) evaluated the efficacy of a polyherbal mouthwash containing hydroalcoholic extracts of *Zingiber officinale, Rosmarinus officinalis* and *Calendula officinalis* (5% v/w) and compared it with chlorhexidine (n=20) and placebo (n=20) mouthwashes in patients having gingivitis. Patients were instructed to use the mouthwash two after breakfast and dinner for 30 seconds for two weeks. Gingival and plaque indices were assessed using MGI (modified gingival index), GBI (gingival bleeding index) and MQH (modified Quigley and Hein index) scores at baseline, day 7 and day 14 of the trial. In the treatment groups, MGI, GBI and MQH scores were significantly lower when compared with those of the control group on day 7 as well as day 14 of the trial. As a result, there was no significant difference found between the polyherbal mouthwash and chlorhexidine groups, at both the follow-up days (day 7 and day 14). Polyherbal mouthwash was considered safe as there was no report of adverse reactions after use. With this, it can be said that 5% Polyherbal mouthwash containing hydroalcoholic extracts of *Z. officinale, R. officinalis* and *C. officinalis* was proved effective in the treatment of gingivitis with its efficacy comparable to that of chlorhexidine mouthwash.[23]

In harmony with this, Javed M, et al. (2009) studied the effect of aqueous extract of *Zingiber officinale, Carum apticum, withania somnifera, Trigonella Foenum Graecum, Silybum marianum, Allium sativum* and *Berberis lyceum* combined

together, on the growth performance of broiler chicks showing the positive effect of broiler growth indicating the nutritive effect of this extract.[24]

Spelman K et al. (2006) conducted an informal survey in which they enlisted multi-component botanical medicines or preparations available in the marketplace and their influence on cytokine function and production. The author considered standardized extraction of whole herb neither isolated nor including multiple herbal formulations. Interleukin-1ß (IL-1ß) and TNF antagonists offer options for the treatment of periodontal disease. When Ashwagandha roots and leaves extract in 80% ethanol was given orally in a dose of 100 mg/kg once a day for 17 weeks in a murine model, it affected IL-1α (increase), TNF (increase) and restored chemotactic activity of carcinogen ochratoxin A (Dhuley et al); whereas, root extract in 70% ethanol; when administered orally, once a day in a dose of 20 mg (intraperitoneal injection) for both 5 days and 10 days, in the murine model showed a change in IL-2 (increase), TNF (decrease), INF-γ (increase) and IL-2 (increase), INF-γ respectively (Davis and Khutan). Nevertheless, when 50% ethanolic extract of Ginger rhizome was administered in-vitro in human cells in a dose of 1×10^4 µg/ml and incubated for 18 hours, they found an increase in IL-6, while, when used in 2×10^4 concentration, it showed an increase in GM-CSF (granulocyte/macrophage-colony stimulating factor) and IL-1β. The difference in the action of Ashwagandha on TNF response could be due to its biphasic dose response.[25]

Sekhar Misra D et al. (2005) performed a study where they studied anti-oxidative potency of oral administration of extract containing Withania somnifera (WS), O. sanctum and Z. officinale in experimentally (swimming) induced oxidative stress in comparison with a standard, potent antioxidant – vitamin E in male Wistar

strain rats. As a result, significant increase was noted in the level of antioxidants in the test group with equivalent potency to vitamin E.[26]

ASHWAGANDHA

Ashwagandha, *Withania somnifera* (L.) Dunal (WS), is also known as the winter cherry or Indian ginseng from Family: *Solanaceae.*[27] The name was given so, due to its characteristic smell which resembles that of a Horse. It is a small, woody, erect, evergreen shrub that grows about two feet (30-150 cm) high, found throughout the drier parts of India. Roots are stout, whitish brown fleshy with simple ovate, glabrous leaves. Leaves in the floral region are placed opposite to each other and smaller in size; whereas flowers are inconspicuous, greenish, or pale-yellow with small, globose, orange-red berries and umbellate cymes in the axillary region of the plant which on maturation, enclose in the persistent calyx. Seeds of WS are yellow in color and reniform. Parts used for therapeutic benefits are a whole plant (including all parts), leaves, roots, green berries, stem, seeds, bark and fruits.[28]

TAXONOMICAL CLASSIFICATION

Kingdom: *Plantae*, Plants;

Subkingdom: *Tracheobionta*;

Super division: *Spermatophyta*,

Division: *Angiosperma*

Class: *Dicotyledons*

Order: *Tubiflorae*

Family: *Solanaceae*

Genus: *Withania*

Species: *Somnifera Dunal*[28]

Synonyms:

Sanskrit: Ashwagandha, Turangi-gandha;

English: Winter Cherry;

Hindi: Punir, asgandh;

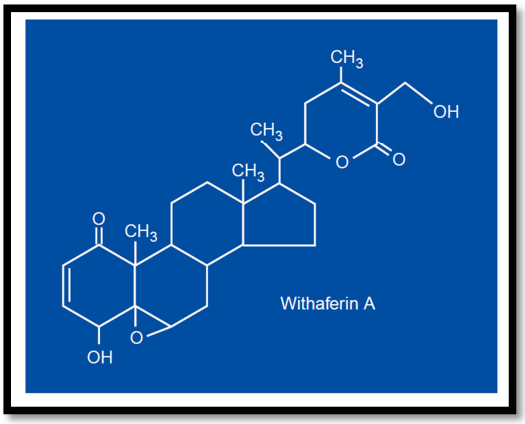
Karnataka: Viremaddlinagadde, Pannaeru, aswagandhi, Kiremallinagida;

Goa: Fatarfoda[28]

The biologically active biochemical constituents found in the WS are alkaloids, steroidal compounds, Withaferin A (WA), Withasomniferin A, Withanolides A, Withasomidienone, Withasomniferols A-C, Withanone, Saponins, and Withanolides with glucose at carbon [27].

WA is chemically characterized as "4b,27- dihydroxy- 5b-6b-epoxy-1-oxowitha-2, 24-dienolide", and is one of the key active principles isolated from the plant. (Gupta GL et al. 2007)[61] WS belongs to the class of drugs Adaptogens or vitalizer causing adaptive reactions to the disease. These are glycosides or alkaloids components of the herb which helps in normalizing pathological effects of the disease.[28]

Chemical structure of Withaferin A:



[28]

The leaves of the plant also showed the presence of 12 withanolides (a group of C28 steroids), alkaloids, many free amino acids, glycosides, chlorogenic acid, glucose, condensed tannins, and flavonoids (Khare, 2007). It is believed that the composition of leaves varies according to the habitat it belongs to. Withanolides have the characteristic feature of containing a "6-membered lactone ring in the 9-carbon atom side chain". While, Withaferin A is a steroidal lactone, the most important withanolide, isolated from the leaf and dried root extract of WS. It has the property of thermostability and water insolubility in addition to slow inactivation at pH 7.2. It is administered in the suspension form. For the separation of WA, the constituents of leaves are extracted with cold alcohol; the extract is then purified, dried and finally crystallized from alcohol.[29]

Ashwagandha was selected based on its immunomodulatory, anti-inflammatory, anti-bacterial and antioxidant properties.[12] As per our knowledge, there are various clinical trials conducted in the medical field not particularly in dentistry to support the animal research done using Ashwagandha in the treatment of anxiety, inflammatory disorders like arthritis and neurological disorders like Parkinson's disease to reduce their symptoms. This pharmacological activity was attributed to

bioactive metabolites withaferin A, withanolides and other alkaloids present in the Ashwagandha. Ashwagandha leaves were selected for the extract preparation as studies showed that withaferin A, present in the leaf extract shows chief anti-inflammatory action of an Ashwagandha similar to commercially available NSAIDs.[12]

Active constituents:

Sandipan Chaterjee et al. (2010) did metabolic profiling of crude extracts of leaves and roots of WS, using NMR (Nuclear magnetic resonance) and chromatographic techniques (HPLC – high-performance liquid chromatography and GC MS – gas chromatography-mass spectrometry). They found a total of 62 major and minor primary and secondary metabolites from leaves and 48 from roots, out of which 29 were common to both. According to this study, Withaferin-A and Withanone are the major metabolites present in the leaf whereas Withanolide A and Withanone are majorly present in the root. In the aqueous-methanolic fraction, the quantity of metabolite in leaves was much higher than that in roots. Nevertheless, ^{1}H and ^{13}C signals clearly indicated the presence of Withaferin A along with withanone as a major metabolite in $CHCl_3$ partition of leaves.[30]

Antibacterial activities:

Abul Qais F et al. (2018)[31] established the inhibitory effect on bacterial respiration along with disruption of membrane integrity and permeability. It was found that WS-AgNPs inhibited the growth of pathogenic bacteria (gram-positive and gram-negative bacteria – *Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa* and *Salmonella typhimurium*) at 16 μg/ml. At sub-minimum inhibitory concentration, there was about 50% inhibition in biofilm formation (validated by light and electron microscopy). WS-AgNPs also eradicated

the biofilms at varying levels at higher concentrations. Accompanied by significant inhibition in bacterial respiration. This could be due to the interaction of WS-AgNPs with test pathogen causing the disruption of the cell membrane leading to leakage of cellular content. WS-AgNPs caused oxidative stress inside bacterial cells triggering microbial growth inhibition and disruption of cellular functions. This was revealed due to the production of intracellular reactive oxygen species.[31]

Halkai KR (2017)[32] formulated silver nanoparticles (AgNPs) using fungi obtained from Ashwagandha leaves and determined the antibacterial efficacy against *Porphyromonas gingivalis, Bacillus pumilus* and *Enterococcus faecalis*, by agar well diffusion method (measuring the zone of inhibition) against *B. pumilus, E. faecalis* and *P. gingivalis.* Study groups were divided depending on the agents used as follows: AgNPs: A (AgNPs - 20 μl), B (AgNPs - 40 μl), C (AgNPs - 60 μl), D (AgNPs - 80 μl), E (AgNPs - 100 μl), F (0.2% chlorhexidine), G (2% chlorhexidine), H (Ampicillin) and I (sterile distilled water). Results showed that AgNPs were as effective as CHX and positive control ampicillin proving efficient antibacterial efficacy of AgNP.[32]

Marslin G et al. (2015)[33] assessed the antimicrobial efficacy of a cream formulation of silver nanoparticles (AgNPs). They were biosynthesized using an aqueous extract of WS leaves, as it promoted efficient green synthesis of AgNPs when fruits and root extracts were compared. Authors compared Cream formulations of AgNPs and AgNO3 for their antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli,* and *Candida albicans.* HPLC analysis of AgNPs discovered that in the nanoparticles, there was selective trapping of some of the phenolic compounds from the WS leaf extract. based on their characteristic UV-vis spectra, these compounds were identified as luteolin-7-

glucoside catechin and p-coumaric acid. The antimicrobial activity of AgNPs can be due to damage to bacterial cellular proteins, blockage of microbial respiratory chain system, disruption of cell membrane and penetration of nanoparticles into the bacterial cytoplasm. Results showed that with the cream of AgNPs, the number of colonies of all the tested pathogens was significantly reduced compared to that of AgNO3. Gram-negative bacteria (P. *aeruginosa*-31 times, *P. vulgaris*-43 times, *E. coli*-28 times and *A. tumefaciens*-13 times) showed a higher reduction in CFUs (colony forming units) on the treatment with AgNPs than AgNO3.[33]

Singariya P et al. (2012)[34] evaluated antibacterial and antifungal effects of WS extracts using different solvents and parts of the plant (hexane, toluene, isopropyl alcohol, acetone and ethanolic extracts of root and stem) with disc diffusion and serial dilution methods against bacterias like *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Raoultella planticola, Enterobactor aerogens* and fungi - *Candida albicans* and *Aspergillus flavus*. As a result, when Isopropyl alcohol, acetone and toluene were used as solvents for the extract preparation, they showed the highest activity against the pathogens showing the broad-spectrum bioactive nature of WS.[34] Thanwar Mayuri conducted a study to evaluate anti-bacterial properties of Ashwagandha leaves using the disc diffusion method on bacterial pathogens like *P. aeruginosa*, *E. coli* and *S. aureus* using different solvents extract preparation. The author found that acetone and methanol extracts showed the highest antibacterial activity followed by the lowest activity in ethyl acetate.[35] This protective activity of WA against various bacteria could be related to activation of macrophage function due to associated increase in phagocytosis along with the intracellular killing of peritoneal macrophages.[66]

Anti-inflammatory activities:

Chandra S et al. (2012)[27] conducted an in vitro study to evaluate the anti-inflammatory effect of ashwagandha extract in comparison with diclofenac sodium against the denaturation of protein (egg albumin). Results showed concentration-dependent inhibition of denaturation of protein by WS greater than that of diclofenac sodium. However, viscosity was decreased in the WS extract-treated group supporting inhibition of protein denaturation. The author attributed these anti-inflammatory effects to the alkaloid and Withanolide contents of Ashwagandha.[27]

Min K. et al. (2011) treated murine microglial cells with withaferin A and observed inhibition of COX-2 mRNA and protein expression and PGE2 production being the reason for the anti-inflammatory property of ashwagandha.[71] Anbalagan et al. studied the action of oral administration of powdered root of WS in rats, when given one hour prior to the induction of inflammation, for three days daily in comparison with phenylbutazone as a positive control. Results showed a considerable reduction in inflammation after WS treatment. Whereas, Immuno-electrophoresis of Acute phase reactants of the blood showed a decrease in the concentration of serum proteins like α2-glycoprotein, major acute phase α1- protein and pre-albumin (normally found in inflammation) in the WS group in contrast to Phenylbutazone. Similar to this, WS also showed a reduction in pain in patients having osteoarthritis.[28]

Immunomodulatory:

Chandran U et al. (2017)[36] investigated immune modulation mechanism for WS and identified five bioactive constituents that are capable of regulating 15 immune system pathways through 16 target proteins by bioactive-target and protein-protein interactions. The study also discloses the potential of the "withanolide-

phytosterol combination" to achieve effective immunomodulation and seven "novel bioactive-immune target combinations". Twenty-two compounds were obtained as root bioactives of WS. The eight bioactive that showed human target interaction were "somniferine, nicotine, choline, 2,3-dihydro withaferin A-3beta-O-sulfate, beta-sitosterol, daucosterol, withaferin-A and withasomniferol-A". Fourteen bioactives in the network did not give human target interaction. The effect was not due to a linear interaction rather it involved a network of interactions involving multiple BA (botanical bio-actives), their TAR (targets), and TAR's interacting partners. Though Withanolides are the most explored compounds of WS, in vitro and in vivo studies revealed the involvement of WA in the suppression of the NF-κB pathway. The five BA having immunomodulatory potential identified through "network pharmacology" were "2,3-dihydro withaferin A-3beta-O-sulfate, beta-sitosterol (immunomodulatory activity and excellent safety profile), daucosterol, withaferin-A, and withasomniferol-A". NEP (Network ethnopharmacology) data provided the importance of the combination of withanolides and phytosterols to get better immunomodulation.[36]

Uddin Q et al. (2012)[29] stated that in animal models (in mice), WS extract (specifically WA) prevented myelosuppression, increased Hb concentration, platelet count, RBC count and body weight, reduced leucopenia and affected both B and T lymphocytes whereas withanolide E specifically affected T lymphocytes.[29]

Grover A et al. (2010)[37] in their study, rationalized the ability of withaferin A to inhibit NF-κB activation by disruption or inhibition of active NEMO/IKKβ (NF-κB Essential Modulator/ IκB kinase) complex formation using molecular docking. However, results from the molecular dynamics simulations in water showed that the trajectories of the protein complexed with WA and native protein were stable over a significantly long time period (2.6 ns).[37]

Anti-oxidant activity:

Sravya MN, et al. (2019)[38] systemically administered herbal antioxidant (Oxitard™) in smokers having chronic periodontitis to assess anti-oxidant properties by analyzing serum procalcitonin levels. Positive results on GI, PPD and CAL were demonstrated at 3-month follow-up. Patients were divided into Group A and B. Group A was comprised of twenty patients receiving Oxitard™ – two capsules two times a day for 3 months after scaling and root planing. Each capsule contains extracts and powder with oil. The extracts contained "Amra (*Mangifera indica*), Ashwagandha (*Withania somnifera*), Gajara (*Daucus carota*), Yashtimadhu (*Glycyrrhiza glabra*) and Draksha (*Vitis vinifera*)". The powder comprised of "Amalaki (*Emblica officinalis*) 141 mg, Lavanga (*Syzygium aromaticum*) 29 mg, Yashada bhashma 2.5 mg, and the oil is made up of Godhuma (*Triticum aestivum*) 6.5 mg. Whereas, Group B included twenty patients undergoing SRP alone. The intragroup comparison showed statistically significant improvement in both the groups including all the assessed variables ($P < 0.001$). On inter-group comparison, there was a marked improvement in both the clinical and biochemical parameters in Group A than Group B ($P < 0.001$). With respect to secondary outcome measures, the intergroup comparison showed improvement in all the parameters in Group A, along with the improvement in serum ProCT ($P < 0.0001$)[38]

S. Sivamani et al. (2014)[11] conducted a study in adult zebrafish on inflammation (stainless steel implant - SSI induced). Fish were divided into four groups – control, SSI without treatment, SSI + WS and SSI + ibuprofen, consisting of 6 each and they were assessed for reduced inflammation (histopathology), local apoptosis (fluorescent quantification) and reverse transcriptase-polymerase chain reaction (RT-PCR) of inflammatory genes. Results concluded that the anti-

inflammatory activity of WS when injected intra-muscularly, might be due to rich flavonoids and phenolic acids as WS (TLC separated portion of supernatant) caused a significant inhibition of TNF-α in the adult zebrafish.[11] As stated by Uddin Q et al (2012)[54] WS also showed an increase in free radical scavenging enzymes like superoxide dismutase (SOD), catalase (CAT) and general peroxidases (GPX) activity in rat brain.[29]

Wound healing**:**

Sheoran S et al. (2020)[39] treated toe gangrene using the ayurvedic mode of treatment (in Shalya tantra outpatient department) for the possibility of management using Ashwagandha Patra Kalka (WS leaf paste) instead of amputation and found improvement in its healing. A 60-year-old male patient, having Vatapitta Prakriti, came with the complaint of a non-healing ulcer at the right big toe associated with severe pain and purulent discharge for 45 days. On local examination, an ulcer was observed over the dorsum of the right toe, measuring about 2.5×2.0×1.0 cm in dimension having irregular margins. There was a presence of slough at the floor of the ulcer with inflamed margins and induration of the surroundings along with the presence of raised local temperature and tenderness on palpation. The patient was treated with the "Panchavalkala kwatha for Dhawana + Ashwagandha Patra Kalka for Lepa" using an insufficient quantity of 1/3rd of the fingerbreadth and was applied over the wound once daily for 28 days. For the initial three days, tablet tramadol – 100 mg one tablet twice daily was continued. Additionally, Vatagajankusha rasa 250 mg one tablet was prescribed two times daily after food for the initial seven days. Complete wound healing was detected by the end of 4th week. There was minimum scar formation post-wound healing. This could be due to Twachya and Vranya

properties of Madhura rasa of Ashwagandha which are essential to maintain the normal color of the skin and minimum scaring.[39]

V Khedgikar et al. (2013)[40] administered WA orally to osteopenic ovariectomized mice and found an increase in osteoprogenitor cells of the bone marrow along with increased expression of osteogenic genes. It was also observed that withanolide and WA acted as a natural proteasomal inhibitor (PI*) and bound to specific catalytic b subunit of the 20S proteasome showing its inhibition within 3 hours (maximum inhibition at 6 hours). Following WA treatment, there was a decrease in osteoclast number directly due to a decrease in expression of tartarate-resistant acid phosphatase and receptor activator of nuclear factor kappa-B (RANK). There was an indirect decrease in the osteoprotegerin/RANK ligand ratio. The authors demonstrated that WA induced osteoblast differentiation by inhibiting the proteasomal Smurf2 expression and degradation of RunX2 protein. On pre-treatment with WA, expression of BMP2 significantly increased.[40]

Javed M et al. (2009)[24] conducted a study to find the effect of *Z. officinale, Carum apticum, W. somnifera, Trigonella Foenum Graecum, Silybum marianum, Allium sativum* and *Berberis lyceum,* on the growth performance of broiler chicks. They were divided into four groups of 60 chicks each (A, B, C and D). It was observed that the combined effect of B. lycium and W. somnifera showed better outcomes rather than when used individually. Aqueous extracts of these plants were mixed with water in dilutions of 5ml/L (group B), 10 ml/L (group C) and 15 ml/L (group D) while group A served as a control. WS has been reported to play a key role in decreasing blood sugar levels, serum cholesterol levels, stress-induced gastric indigestion and ulcers.[24]

Anti-cancerous:

Uddin Q et al. (2012)[29] described the anti-tumor activity of Ashwagandha due to the presence of WA, withanolide D & E, active constituents of WS. They showed significant activity against human epidermoid carcinoma (nasopharynx), human larynx carcinoma cells (at metaphase) and embryonic chicken fibroblast cells causing mitotic arrest. Isolated WA showed radio-sensitizing effects along with the SER ratio (sensitizer enhancement ratio) of 1.5 for in-vitro cell killing (Chinese hamster cells, V79).[29]

Gupta GL et al. (2007)[41] demonstrated the chemopreventive effect of WS root extract in skin cancer-induced mice when given before and during exposure to the skin cancer-causing agent. It was observed that, following administration of the extract, levels of reduced glutathione, superoxide dismutase (SOD), catalase (CAT) and general peroxidases (GPX) in the exposed tissue returned closed to normal values. This chemo-preventive activity was thought to be due to the antioxidant or free radical scavenging activity of the extract. WA more effectively inhibited the growth of breast and colon cancer cell lines than doxorubicin. Withanolides also suppressed NF-kappaB activation including inhibition of tumor necrosis factor (TNF), interleukin-1β, doxorubicin and cigarette smoke condensate. As inducible and constitutive NF-kappaB activation was blocked by withanolides, Suppression was not specific to cell type.[41]

Other actions**:**

Uddin Q et al. (2012)[29] also described other actions of WS extract like cognition-enhancing and memory-improving effects, relaxant and antispasmodic effects, anti-aging, anti-hyperglycemic (decrease in pancreatic islet cell SOD activity)

effects, reduction in macrophage chemotactic activity, suppression in morphine withdrawal jumps and hepatoprotective activity.[29]

Prabhakara Reddy Nagareddy and M. Lakshmana (2006) studied *Withania somnifera* extract (ethanolic), which contained oestrogen-like withanolides for anti-osteoporotic activity. It is widely used as it has anti-inflammatory, immunomodulatory, anti-tumour, anticoagulant, anti-rheumatic and anti-osteoarthritis activity. Along with this, it is also known to have a positive influence on the endocrine and central nervous systems. According to this study, it can be said that WS shows positive effects on the endocrine system, which may have increased bone formation and reduced resorption of bone minerals into the systematic circulation.[42]

Many pharmacological studies have been conducted to explore the properties of Ashwagandha to authenticate its use as a multipurpose medicinal agent. Its anti-inflammatory properties have been investigated to validate its use in inflammatory arthritis. There are many animal-stress studies have been performed to investigate its use as an antistress agent. Several studies have examined the antitumor and radio-sensitizing effect of WS. (Mishra LC, 2000)[28]

Cytotoxicity:

According to the safety assessment study done by Patel SB et al. (2016)[43] on Wistar rats by oral administration of standardized WS extract for up to 28 days, it was observed that maximum of 2000 mg/kg body weight can be administered without causing any adverse or toxic effects on body weights and physical/ behavioral observations.[43]

GINGER

Ginger, scientifically known as *Zingiber officinale Roscoe* (family Zingiberaceae), is a vital plant with several remedial and nutritional principles. The origin of the word "ginger" can be traced back to the Sanskrit/Pali word "singabera," meaning "shaped-like a horn" based on its appearance. It was well known to Indian and Chinese medicine and was referred to as "maha aushadhi" (the great medicine) in Vedic literature. It is used predominantly as a spice, natural additive, and in herbal remedies. Customarily, ginger is used in complementary and alternative medicine (CAM) of different countries such as Africa, China, India, and Arabian ethnomedicine to cure various ailments such as nausea, vomiting, headache, allergies, asthma, cough, and pain, to name a few. It is cultivated through rhizomes and grows well in tropical and subtropical areas, perennially. The rhizomes are full of aroma and have thick lobes with ring-like scars, growing up to a size of 30-90 cm.[44]

Although the increase in offensive acid-pepsin secretion was observed in this study, the increase in defensive mucin secretion was sufficient enough to protect against experimental ulcers. Ginger was shown to significantly scavenge superoxide and hydroxyl radicals and inhibit lipid peroxidation.[45]

TAXONOMIC CLASSIFICATION

Kingdom – Plantae – plantes,

Subkingdom – Viridiplantae

Infrakingdom – Streptophyta

Superdivision – Embryophyta

Division – Tracheophyta

Subdivision – Spermatophytina

Class – Magnoliopsida

Superorder – Lilianae

Order – Zingiberales

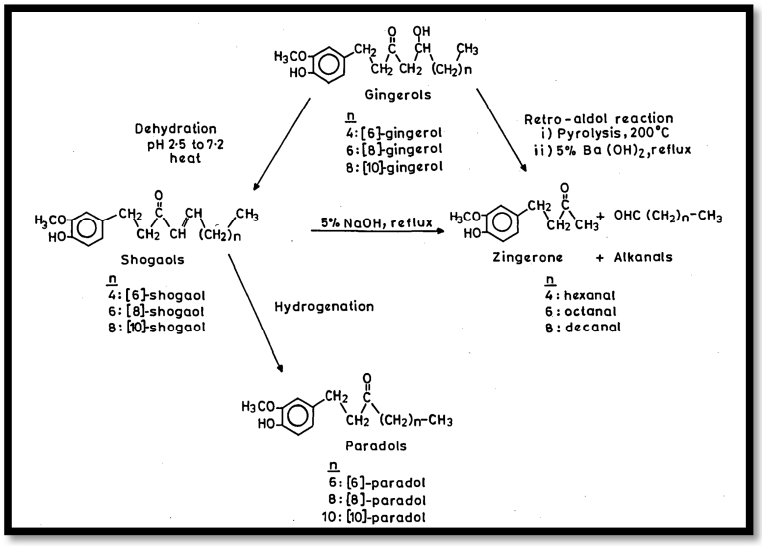
Family – Zingiberaceae

Genus – *Zingiber Mill.*

Species – *Zingiber officinale Roscoe*[46]

Composition:

Ginger's medicinal properties and physiological actions are primarily attributed to specific bioactive constituents of plants explicitly alkaloids, flavonoids, tannins, and phenolic products. The Greek physician Galen believed that ginger was a purificant of the body and can treat diseases caused by bodily imbalances. The main active ingredient present in ginger includes phenolic compounds (gingerol and shogaol) sesquiterpene hydrocarbons and oleoresins. (Rashmi KJ et al. 2016)[44]



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Active constituents:

Studies on rhizome extracts of ginger showed that active gingerols from ginger may convert to paradol, zingerone and shogaols like compounds out of which 6- shogaol and 6-gingerol were found to be associated with antitussive, hypotensive, analgesic, antipyretic and anti-carcinogenic properties.[47]

Shubha Ratna Shakya (2015) mentioned in her review the medicinal uses of ginger (Z*ingiber officinale*). Ginger is considered now as a drug of choice for nausea and vomiting and is also used in pregnancy-related morning sickness. It acts as a natural pain reliever in rheumatoid arthritis, osteoarthritis and also as an anti-inflammatory agent. It has a wide range of actions starting from curing ulcers to preventing heart attack and stroke. Ginger has shown to have several nutritive, ethnomedicinal values and many medicinal properties such as cardio-protective, anti-inflammatory, anti-microbial, anti-oxidant, anti-cancer properties.[14]

Antibacterial activities**:**

Sana'a MA and Ahmed MA (2017) showed that concentrations of alcoholic extract had more antibacterial activity as inhibition zones were having higher mean values for *A. a* than the concentrations of aqueous extract, this could be due to 1) the amount of active component (10-gingerol – primary active phenol in ginger acting against anaerobic periodontopathic bacteria) was higher in the alcoholic ginger extract (higher concentration). 2) the polarity of the solvent (ethanol alcohol) having great ability to dissolve biologically active constituents of ginger.[48]

Santo Grace U and Sankari M (2017)[49] compared effects of ethanolic extract of Z*ingiber officinale* on *S. aureus* and *E. faecalis*. They found that ethanolic extract is more efficient in gram-positive organisms (stronger inhibitory effects). Malu et al. suggested that inhibition of the bacterial growth activity is dose-dependent and could be attributed to the chemical properties of ginger constituents – sesquiterpenoid, zingiberene. Pankaj et al. (2017) found that boiling temperature treated crude ginger extract lose its anti-microbial activity (against *K. pneumonia, E. coli, S. aureus*).[49]

Nikolić M et al. (2014)[50] evaluated antibacterial (by microdilution method) and anti-biofilm activity (by crystal violet assay) of ethanolic extract from the rhizome of *Zingiber officinale* in-vitro. MIC and MBC values were in the range from 0.0024 to > 20 mg/ml. The percentage of *P. aeruginosa* biofilm reduction was 10.5 for the 20 mg/ml concentration of investigated extract showing moderate anti-biofilm activity.[50]

In accordance with this, Yahya et al. (2013) found out that the ethanolic extract of Z. officinale inhibited *P. aeruginosa* biofilm formation under both aerobic and anaerobic environments.[33]

Han-Shin Kim and Hee-Deung Park (2013) evaluated Ginger's (Z*ingiber officinale*) ability to inhibit *Pseudomonas aeruginosa* PA14. By chemical analysis and confocal laser scanning microscopy, it was confirmed that ginger extract decreased the production of extracellular polymeric substances. A static biofilm assay demonstrated that, on the addition of the ginger extract to the culture, the biofilm development was reduced by 39-56% and it had lower EPS production along with the swarming motility on agar plates. the detachment efficiency for biofilm cells formed with ginger extract (79%) was higher, suggesting their loose attachment on the surface. It inhibits biofilm formation through reduction of cellular c-di-GMP along with alteration of its phenotype without causing any alteration in QS without affecting the growth of the bacteria avoiding the development of antibiotic resistant species.[51]

Miri Park, Jungdon Bae and Dae-Sil Lee (2008) in their study isolated and elucidated effective anti-bacterial agents from the ginger rhizome against periodontal oral pathogens. Products of gram-negative bacteria like *Porphyromonas* and *Prevotella* species which are obligate anaerobes and principal oral pathogens, suppress host defense mechanisms and destroy periodontal tissues, leading to the loss of alveolar bone and teeth in the pathogenesis of human periodontitis. This study also showed that the antimicrobial activity of [10]-gingerol was higher than that of [12]-gingerol. Though both 10- and 12- gingerol demonstrated potent anti-bacterial activities in-vitro (against anaerobic periodontopathogens).[16]

Caiazza et al. (2007) demonstrated an inverse relation of biofilm formation and swarming motility of PA14 via Pel polysaccharide and flagella reversal. A probable mechanism of biofilm inhibition by ginger extract could be the modulation of certain proteins leading to degradation or lowering the synthesis of c-di-GMP. Ginger extract

is considered a broad-spectrum biofilm inhibitor as it can directly be used to reduce virulence factors or to treat infectious bacteria and their biofilm.[51]

Anti-inflammatory activities:

Pallavi Menon et al. (2020)[47] conducted a study to assess anti-inflammatory and analgesic properties of Ginger rhizome in patients with chronic generalized periodontitis, where capsules containing ginger powder (400mg) were given in 10 patients three times a day, for three days compared to Ibuprofen (400 mg) as a standard NSAID drug as an adjunct to open flap debridement. Results displayed that at the eighth hour, the mean value of the score of pain in the ginger group was higher than the ibuprofen group, although there was no statistically significant difference between the scores at intervals of 1 hour, day 0 and day 7 ginger was having comparable effects on reducing inflammation (MGI) and pain management (VAS score) on comparison between two groups showing equivalent effectiveness of ginger as an anti-inflammatory and analgesic drug.[47]

Javid AZ et al. (2019)[52] where they evaluated the result of supplementation with 2 gm ginger tablets (4 tablets of 500 mg each) orally for 8 weeks in Type 2 DM chronic periodontitis patients along with NSPT and found effective improvement in inflammation, oxidative and periodontal status.[52]

To determine the anti-inflammatory action of ginger on the improvement of clinical symptoms of rheumatoid arthritis, Haghpanah et al. (2015) instituted mucoadhesive containing ginger extract and observed a significant reduction in the intensity of pain in the treatment group (visual analog scale). Aktan et al. (2006) discovered that ginger partially inhibited nitric oxide synthase (iNOS) activity along with the reduction of the NO production in macrophages via attenuation of NF-kappa-

B mediated iNOS gene expression. In another study, Srivastava et al. in 1992 studied the effects of ginger on rheumatism or musculoskeletal disorders. The author suggested that the anti-inflammatory activity can be due to prostaglandin and leukotriene suppression along with dual inhibition of eicosanoid biosynthesis.[44]

Flynn and Rafferty demonstrated for the first time that some of the constituents of ginger act as functional dual inhibitors of arachidonic acid metabolism (COX and LOX) which include [6]-gingerol, shagaol, gingerdione and dihydroparadol. This might be one of the reasons why high doses of ginger extract do not show the side effects often observed with NSAIDs (non-selective COX inhibitors).[53]

Immunomodulatory:

Yang X et al. (2021)[54] investigated NGP - A neutral ginger polysaccharide fraction, isolated from ginger including its primary structures and immunomodulatory activities. It had a low molecular weight of 6305 Da and it was principally consisted of glucose (93.88%) together with minor levels of galactose (3.27%) and arabinose (1.67%). NGP also displayed a remarkable immunological activity by significantly enhancing the proliferation of macrophages without cytotoxicity and increased the production of immune substances like TNF-α, NO, IL-6 and IL-1β. They found the secretion at the concentration of 200 μg/mL was 1496.71 pg/mL, 29.41 μM, 1889.83 pg/mL and 44.30 pg/mL for each substance, respectively. The results indicated that NGP could be a potential immune agent and might provide meaningful information for further chain conformation and immune mechanism research.[54]

Anti-oxidant activity:

Ginger is a rich source of antioxidants that acts by scavenging superoxide anion, hydroperoxide and hydroxyl free radicals. It also prevents lipid peroxidation (LPO) and inhibits NO synthesis. Compounds, such as 6 dehydroshogaol, 6-shogaol, and 1-dehydro-6-gingerdione are the active ingredients known to possess potent antioxidant activity due to the presence of unsaturated ketone in their structures.[44]

The results of the study done by Tohma H, et al. (2017)[55] supported the finding that the ethanolic extract had generally better antioxidant activity (determined using ferric thiocyanate methods) than aqueous extract in all assays. HPLC-MS/MS analysis showed that pyrogallol p-hydroxybenzoic acid, ferulic acid and p-coumaric acid were more abundant in both extracts, demonstrating effective antioxidant properties and further reducing or delaying the progression of the chronic inflammatory disease.[66] When Shirin Adel and Prakash (2010) analyzed components of ginger like vitamin C, β carotene, flavonoids, polyphenols and tannins, they concluded that maximum antioxidant activity was present in the alcoholic extracts.[55]

Wound healing:

Rahayu KI et al. (2020)[56] determined the effect of ginger extract on the number of neutrophils, fibroblast cells and epithelialization in incision wounds in white mice where they were given oral ginger extract at a dose of 1 g / kg by weight. Results showed a reduction in the number of neutrophil cells, increase in the number of fibroblast cells, and increase in epithelialization of incisional wounds mainly due to inhibition of cyclogenese activity followed by a decrease in prostaglandin synthesis causing a decrease in inflammatory mediators further reducing the number of neutrophils by its active constituents like gingerol.[56]

This was in support of the study done by Bhagavathula N et al. in 2009 where hairless rats were treated with topical application of 10% curcumin, 3% ginger and using a combination of both. They found that healing was more rapid in the skin of rats that had been pretreated with either curcumin or ginger extract alone or in combination without any side effects like skin irritation. It was also observed that there was increased collagen production along with the decrease in matrix metalloproteinase-9 production in recently healed skin from rats treated with the herbal preparation.[57]

Anti-cancerous;

Tumorigenesis and its progression are multi-step processes controlled by genetic and environmental factors. Ginger can control tumor growth and proliferation by inducing apoptosis, upregulation of tumor suppressor genes and inhibition of angiogenic factors (vascular endothelial growth factor). Investigations have shown that 6-gingerol, 6-shogaol, 6-paradol, zingerone and zerumbone possess major antitumor properties, where, 6-shogaol causes apoptotic death in human prostate cancer lines and 6-gingerol inhibits NF-κB activation and activates the G0-G1 cycle through downregulation of cyclin D1. Gingerol activity has been found to be effective against breast, colon, prostate cancer and promyelocytic leukemia. On investigation of Dietary ginger using albino rats, results showed radioprotection at the biochemical level which could be due to whole-body exposure to fast neutrons. Pre-treatment with ginger extract before radiotherapy was found to decrease glutathione reductase, glutathione peroxidase enzyme activities. Similarly, alcoholic extracts of ginger (250 mg/kg) when administered orally, once a day for 5 consecutive days before exposure to gamma radiation, they showed a reduction in the severity of radiation sickness symptoms.[44]

Recently, when studied in a mouse skin tumorigenesis model, ethanol extracts of ginger were shown to have antitumor promoting effects. The animals pretreated with ginger extracts showed substantially lower tumor body burdens when compared with non ginger treated controls. Aqueous extracts of ginger were found to be cytotoxic to lymphoma ascites tumour cells in culture. Ginger extracts, gingerol and shogaol were found to be mutagenic in the presence of a rat liver metabolic activation system when tested in a *Salmonella*/microsome assay. In contrast, zingerone was found not to have mutagenic properties and it suppressed the mutagenic activity of gingerol and shogaol in a dose-dependent manner.[58]

Other actions;

Phenolic and flavonoid compounds present in Ginger are considered as neuroprotective. It was found that 6-shogaol caused inhibition of microglial matter and showed neuroprotective properties in transient global ischemia by Ginger and there was an increase in the mucin secretion by its constituents, in turn, causing ulcer prevention. Chamani et al. (2011) suggested that when the ginger extract was applied systemically, it increased the rate of salivation due to the direct parasympathomimetics effect on the post-synaptic M3 receptors and repressed presynaptic muscarinic auto-receptors. Several in vitro studies proposed that ginger extracts inhibit platelet aggregation. In a study on 12 healthy subjects at recommended doses, it was observed that ginger did not significantly affect the clotting status, the pharmacokinetics, or the pharmacodynamics of warfarin.[44] Whereas, in a systematic review done by Terry et al., in 2011, assessed the worth of ginger in the management of pain and presented that the current evidence supported the anti-inflammatory and analgesic effects of ginger, responsible for the decrease in the inflammation and pain perception in conditions like osteoarthritis.[47]

Reinhard Grzanna et al. in 2005[53] in their review attributed PG inhibiting activity of the ginger to the hydroxymethoxyphenyl compound's (HAPC) presence. They stated that prostaglandin synthesis was suppressed by Ginger through nonselective inhibition of COX-1 and -2, sharing similar properties to NSAID drugs. Although it is a more potent COX-1 inhibitor, it showed protective effects against the inhibition of COX-1 (toxicity associated). Some of the constituents present in ginger inhibit PG synthesis, as well as LT synthesis as both, are derived from arachidonic acid. In chronic inflammatory conditions, proinflammatory cytokines are up-regulated, either by inhibiting their production or blocking action which can achieve successful disease control. According to the studies conducted on human synoviocytes and chondrocytes using ginger extract, they showed inhibition of the expression of TNF-α in synoviocytes as well as inactivated chondrocytes at the transcription and protein levels along with gene encoding chemokines – MCP-1 (monocyte chemotactic protein) and IFN-γ inducible protein -10. To contradict these findings, Frondoza et al. conducted a preliminary experiment where they observed that 6-gingerol weakly inhibits LPS-induced cytokine induction in monocytic THP-1 (human leukemia monocytic cell line) cells. On the pre-treatment with ginger extract using THP-1 cells, Grzanna et al. detected reduction or completely prevented induction of gene, showing extended anti-inflammatory properties of ginger to other cell types as well.[53]

Powdered ginger rhizome has been used traditionally as a remedy in the treatment of peptic ulceration although ginger promotes gastric secretions and increases gastrointestinal propulsion. Its high doses might act as a gastric irritant. It was observed that fresh ginger of about 6 g or more might be a reason for "a significant increase in the exfoliation of gastric surface epithelial cells in human

volunteers". Its anti-motion sickness property could be a result of its "effects on the gastrointestinal tract" rather than that "on the central nervous system". 6-gingesulphonic acid, present in the ginger showed more potent anti-ulcer activity (because of effective thromboxane synthetase inhibition) and weaker pungency than 6-gingerol and 6-shogaol. Ginger also showed significant scavenging of hydroxyl and superoxide radicals and inhibition of lipid peroxidation. (Goyal RK et al., 2002)[45]

Bliddal et al. in 2000 and Haghighi et al. in 2005 weighted up the effects of ginger and ibuprofen on the subjects with osteoarthritis and determined that the difference between effects of both on pain was statistically not significant.[47]

Adverse effects and Cytotoxicity:

Ginger might cause mild gastrointestinal side effects like irritation, heartburn or diarrhea.[44] Weidner MS and Sigwart K in 2000 detected that when EV.EXT 33 (a specialized patented standardized ethanolic extract of dry rhizomes of ginger) was given to pregnant rats during the period of organogenesis, it resulted in neither maternal nor developmental toxicity with daily maximum dose of 1000 mg/kg body weight.[59]

Alkaline Phosphatase Levels in Gingival Crevicular Fluid:

Prihastuti CC et al. in 2019[60] evaluated the effect of potato skin extract in the treatment of periodontitis on alveolar bone and serum ALP levels of rats. They divided thirty-five male Sprague Dawley rats into five groups: healthy control, negative control (aqua dest-treated periodontitis) and potato skin extract-treated periodontitis in three different concentrations (25%, 50%, 75%). After 14-days of the treatment, ALP levels on alveolar bone and serum samples from each group were

measured using UV-V is a spectrophotometer. The results showed both alveolar bone ALP and serum ALP levels were lower in potato skin extract-treated periodontitis groups in comparison to the negative control group. Therefore, the study concluded that potato skin extract decreased ALP level, with a concentration of 75% showing the lowest ALP levels of alveolar bone and serum samples, indicating alveolar bone remodeling. Moreover, it also suggested that the ALP level in serum can represent ALP level on alveolar bone tissues in periodontitis.[60]

Durga Bai Yendluri in 2018[18] studied the activity of Alkaline phosphatase in gingival crevicular fluid (GCF) in patients having chronic and aggressive periodontitis with their age and gender-matched healthy controls. There were the following Groups present: Group A - 10 aggressive periodontitis patients with ages ranging from 18 – 22 years. Group B - 10 healthy controls (18-22 years). Group C - 10 chronic periodontitis patients with ages ranging from 35-55 years. Group D - 10 healthy controls (35-55 years). The ecology of the periodontal pocket was determined by GCF, as GCF contains the products of inflammatory response and also determines the growth rate of subgingival microorganisms. It was believed that the ratio of GCF to serum alkaline phosphatase ranged from 6:1 to 11:1 recommending its major origin from local production. According to the author, the increase in alkaline phosphatase in periodontal disease indicated a metabolic reaction of the enzyme against the tissue changes caused by inflammation. The concentration of Alkaline phosphatase was shown to be positively associated with periodontal disease activity, thus can be used in the early detection of gingival and periodontal disease states.[18]

To support the use of ALP as an inflammatory marker, Kapil H et al in 2018 conducted a similar study in which they assessed ALP levels, showing greater levels in the disease state followed by a statistically significant decrease at 6-week follow-

ups post-treatment due to reduction in inflammation. They administered 0.2% Thymoquinone gel, adjunct to scaling and root planing in the periodontitis treatment. Results showed a statistically significant reduction in RAL (relative attachment level), PPD, GI and PI at 6 weeks post-operative evaluation. They even checked the anti-bacterial activity of Thymoquinone, an active component of *Nigella sativa* against *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia*; authors observed that included bacterial strains were found to be highly sensitive against "0.2% Thymoquinone gel" proving the importance of ALP in diagnosing and monitoring inflammation.[6]

Sanikop S et al. (2012)[20] collected GCF from 45 sites, which were divided into three equal groups of healthy, gingivitis and chronic periodontitis samples. The ALP levels were estimated using a semi-autoanalyzer. The difference in the mean ALP levels between the chronic periodontitis group, healthy as well as gingivitis groups was found to be highly significant ($p<0.001$). There were significant correlations existed between ALP levels and gingival index, probing depths as well as clinical attachment levels. Therefore, the author suggested the use of GCF ALP levels as a potential biochemical marker for the detection and progression of periodontal disease. The presence of ALP in the saliva and GCF is usually indicative of inflammation and/or destruction of the periodontal tissues. The level of ALP is positively correlated with the severity of periodontal disease.[20]

Ishikawa and Cimasoni in 1970 stated that the amount of fluid in the gingival sulcus varies according to the severity of inflammation along with the positive correlation of β-glucuronidase to the severity of periodontitis. They also significantly correlated Alkaline Phosphatase levels in GCF to the level of periodontal pocket depth and noticed that ALP levels were three-fold higher than that of serum.[61]

MATERIALS AND METHODS

SOURCE OF DATA:

The study was conducted in the Department of Periodontics, KAHER's KLE V.K. Institute of Dental Sciences, Belagavi. An ethical clearance (Annexure I) was obtained before conducting the study from the Ethical Committee, KAHER's KLE V.K. Institute of Dental Sciences, Belagavi.

A total of 23 patients with moderate chronic periodontitis (AAP 1999 Classification of periodontitis)[62] who gave consent for the study and met the following inclusion and exclusion criteria were included in the study.

INCLUSION CRITERIA:

- Minimum of 20 sound teeth to be present.
- Age – 30 to 55 years of either sex.
- Systemically healthy patients.
- Patients with chronic periodontitis (AAP 1999 Classification of periodontitis)[62] having pocket probing depth > 4mm in contra-lateral quadrants.
- Patients who have not undergone any form of dental treatment (non-surgical or surgical periodontal therapy) in the last 6 months including oral prophylaxis.

EXCLUSION CRITERIA:

- Patients having a systemic disease that could influence the periodontal outcome
- Patients with a history of antibiotics and non-steroidal anti-inflammatory drug use in the past 6 months.
- Pregnant women and lactating mothers.
- Smokers and patients consuming tobacco in any other form.
- Immuno-compromised patients.
- Patients who have a history of any kind of allergy.
- Patients not willing to participate in the study

STUDY DESIGN:

A randomized controlled trial with a split-mouth design was followed. In each patient, two contralateral sites were selected randomly having pocket probing depth of >4mm. Randomization was done using the coin toss method and sites were divided into test and control sites.

- Test sites (n=23): Scaling and Root Planing along with placement of local drug delivery of polyherbal gel containing Ashwagandha and Ginger
- Control sites (n=23): Scaling and Root Planing alone.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) and MINIMUM BACTERICIDAL CONCENTRATION (MBC):

A pilot study was done to determine the MIC of Ashwagandha, Ginger, both combined (Ashwagandha + Ginger) against *Staphylococcus aureus*, a gram-positive, facultative aerobe using serial broth dilution method at KAHER's Dr. Prabhakar Kore Basic Science Research Centre, Belagavi.

MATERIALS:

- Eppendorf tubes
- 96 well plate
- Micropipettes (10µL, 100µL, 1000 µL)
- Brain heart infusion (BHI) broth and agar
- Glass petri plate
- Glass funnel
- Inoculum: *Staphylococcus aureus*
- Stalk solutions of Ashwagandha (*Withania somnifera*) 80mg/ml, Ginger (*Gingiber officinale*) 200mg/ml and both together
- Dimethyl sulfoxide (DMSO)

PROCEDURE:

Ashwagandha leaves and Ginger rhizome (Fig. 1) were dried and sent for authentication and ethanolic extracts preparation (Fig. 2) to KAHER's B.M.K. Ayurvedic Mahavidyalaya, Belagavi. They were dried in glass petri plates to attain a solid consistency. To make a homogeneous solution of extracts they were further dissolved into 5% DMSO for the determination of MIC and MBC.

For all the three stalk solutions [Ashwagandha (*Withania somnifera*) 80mg/ml, Ginger (*Gingiber officinale*) 200mg/ml and both together] similar procedure was followed. The experiment was done using 96 well plate. Ten wells were selected from 96 well plates for the broth dilution method. A total of 100 µL of broth was added to all the 10 wells, in the first well 100 µl of the emulsion was added and serially diluted to required concentrations up to the tenth well. Further, 20 µl bacterial inoculum was added to all the ten wells; separate wells were used for positive and negative controls. The 96 well plate was kept for incubation in a bacteriological incubator for 12 hours and resazurin reagent (20 µL) was added after 12 hours to each well. Wells were observed after 4 hours for possible colour change. Any color change from blue/violet to slight pink/pink/magenta was recorded as MIC of stalk solution. The experiment was done in triplicates. (Fig. 3)

The MIC (minimum inhibitory concentration) was defined as the lowest concentration of Ashwagandha, Ginger and both together where no visible growth of bacteria was seen at the end of 12 hours.

MBC (minimum bactericidal concentration) was also checked using all the dilutions with the help of BHI agar plates in triplicates. From each well 10 µL was pipetted and streaked using nichrome wire loop on the BHI agar plates. The plates were sealed with the paraffin film and were incubated in the bacteriological incubator for 12 hours. At the end, minimum concentration at which bacteria did not show growth was considered as MBC value.

Polyherbal gel was prepared by considering the MIC and MBC results obtained from the above procedures:

MIC and MBC for Ashwagandha was 6.6 mg (Fig. 4a); for Ginger 130 mg (Fig. 4b) and for the combination of Ashwagandha and Ginger, it was 35 mg (Fig. 4c).

PREPARATION OF POLYHERBAL GEL CONTAINING ASHWAGANDHA AND GINGER:

The 5 ml of 2.5% polyherbal gel containing *Withania somnifera* and *Gingiber officinale* extract was prepared in the Dr. Prabhakar Kore Basic Science Research Centre, KAHER's KLE University, Belagavi

MATERIALS:

- Ethanolic extracts of Ashwagandha and Ginger
- Carbopol 940
- PEG 400
- Methyl and propyl paraben
- Distilled water
- Magnetic stirrer
- Digital weighing scale
- 96 well plate
- Pipette
- Filter paper
- Glass measuring cylinder
- Pipettes
- Nichrome loops

PROCEDURE:

Ethanolic extracts (Fig. 2) of Ashwagandha Leaves and Ginger Rhizome were dried in a glass Petri plate to attain a solid consistency. To make a homogeneous solution of extracts, they were further dissolved into diluted DMSO solution so as to use in gel preparation. 1% Carbopol solution was prepared by dissolving Carbopol in water and was kept overnight on the magnetic stirrer to form a homogeneous mixture. Propyl and methylparaben were dissolved separately in water and were then added to the previously prepared Carbopol solution. Both Ashwagandha and Ginger extracts were dissolved in PEG 400 and were further added to the above-mentioned solution. The quantity of extracts to be added was determined based on MIC and MBC values of extracts. Further, the volume was adjusted to 5 ml by adding sufficient water.[28] (Fig. 5)

The pH and viscosity of the prepared gel were observed to be 5.1 and 680 cP (centipoise).

The gel was sterilized in the UV radiation chamber before its clinical use. (Fig. 6)

STEPWISE PROCEDURE USED IN THE STUDY:

CLINICAL EXAMINATION AND PLACEMENT OF POLYHERBAL GEL:

CLINICAL ARMAMENTARIUM: (Fig. 7)

- Surgical drape
- Mouth mask
- Disposable gloves
- Mouth mirror
- Straight probe
- Explorer

- William's periodontal probe
- Tweezers
- Ultrasonic scaler unit with ultrasonic scaling tips.
- Kidney tray
- Cotton rolls
- Gauze pieces
- Glass slab
- Mixing spatula
- Normal saline
- Periosteal elevator Glickman-6 (P24G)
- Polyherbal gel containing Ashwagandha and Ginger

FOR GCF (Gingival Crevicular Fluid) BIOMARKER ANALYSIS:

- Calibrated micro-capillary tubes
- Sterilized micro-centrifuge tubes (Eppendorf tubes)
- Biochemical kit for Alkaline phosphatase (Erba Mannheim Chemicals)

PROCEDURE:

Patients satisfying the eligibility criteria were included in the study. Consent was obtained from the patient before commencement of the procedure (Annexure II). A special proforma was designed for the study to have a systematic recording of all the observations and patient information. The relevant data consisting of details of name, age, sex, chief complaint, past medical history and past dental history was recorded in the special proforma (Annexure III). Clinical examination was performed on a dental chair under standard conditions of light using a mouth mirror, explorer

and graduated William's periodontal probe. In the patients following Eligibility Criteria (Fig 8a), Test and control sites were randomly selected using Coin Toss Method in Contra-lateral quadrants. Clinical and biochemical parameters were assessed for both Test and Control sites at Baseline as well as at 4 weeks after the treatment.

Full mouth scaling and root planning was performed (Fig. 8b). Gingival Crevicular Fluid was collected from the target site (Fig. 9) in both Test (before placement of the gel) and Control group (Fig. 10). Test site was dried first followed by isolation with placement of cotton rolls. After making sure of complete removal of subgingival calculus, polyherbal gel was placed at the test site using Periosteal Glickman-6 (P24G) elevator (Fig. 11). Periodontal dressing was placed on the test site (Fig. 12).

Patients were given instructions regarding maintenance of the oral hygiene such as following modified bass technique except for the region of dressing. They were advised not to use any of the other plaque control techniques and were recalled after 4 weeks for re-evaluation. All clinical (Fig. 13a, 14a) and biochemical parameters were assessed pre-operatively at baseline and 4 weeks post-operatively (Fig. 13b, 14b) by single examiner.

The clinical and biochemical parameters assessed at baseline and at 4 weeks were:

I. Plaque Index (Silness and Löe, 1964)[63]
II. Sulcus bleeding index (Muhlemann H.R and Son S, 1971)[64]
III. Probing pocket depth (PPD)
IV. Clinical Attachment Levels (CAL)
V. Alkaline Phosphatase levels in Gingival Crevicular Fluid

PLAQUE INDEX (Silness and Löe, 1964)[63]:

The Plaque Index is unique among the indices used for assessment of plaque because it ignores the coronal extent of plaque on the tooth surface area and assesses only the thickness of plaque at the gingival areas of the tooth. The fundamental principle of this index is desirability of clear distinction between severity and location of soft debris. It also demonstrated good validity and reliability. However, the subjectivity in estimating plaque is still considered as an issue. Therefore, it was recommended that a single examiner should be trained and used with each group of patients till completion of a clinical trial.

PROCEDURE:

The tooth was dried and examined visually. The explorer was passed across the tooth surface in the cervical third and near the entrance of the sulcus. When no plaque adheres to the point of the explorer the area is considered to have a '0' score. When plaque adheres and form a thin film, a score of 1 is assigned. When deposits are visible, score '2' is given and score '3' is reserved for heavy plaque accumulation. Plaque present on the surface of calculus deposits and on all types of dental restorations in the cervical third is evaluated and included. The distal-facial, facial, mesial-facial and lingual gingival areas of the tooth were examined.

SCORING CRITERIA:

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be recognised only after running the probe around the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and /or on the tooth and gingival margin

CALCULATION:

Each area (distal-facial, facial, mesial-facial, lingual) is assigned a score from 0 to 3, which is considered as the plaque index for the area. Mean score was calculated per tooth by addition of the scores from the four areas of the tooth followed by division with four. This gives the plaque index for the tooth of interest.

SULCUS BLEEDING INDEX (Muhlemann H.R & Son S, 1971)[64]:

The Sulcus Bleeding Index (SBI) is an index for assessment of gingival bleeding. The purpose of this index is to locate areas of gingival sulcus bleeding upon gentle probing and thus recognize and record the presence of early inflammatory gingival disease.

PROCEDURE:

The SBI is based on the evaluation of gingival bleeding on probing which is first sign to occur, gingival contour and gingival colour changes. Four gingival units were scored systematically for each tooth: the labial and lingual marginal gingiva (M units), and the mesial and distal papillary gingiva (P units). The probing of four areas was carried out under proper illumination. The probe was held parallel with the long axis of the tooth for M units and directed towards the col area for P units. After probing was done 30 seconds were allowed to pass before scoring apparently healthy gingival units. The gingiva was dried gently to observe colour changes clearly.

SCORING CRITERIA:

Score	Criteria
0	Healthy appearance of P and M, no bleeding upon sulcus probing. gingiva of normal texture and colour
1	Apparently healthy P and M showing no colour or contour changes and no swelling, but bleeding from sulcus on probing.
2	Bleeding on probing and colour changes caused by inflammation (reddening). No swelling or macroscopic oedema.
3	Bleeding on probing, change in colour, slight oedematous swelling
4	Bleeding on probing and/or colour change OR obvious swelling.
5	Spontaneous bleeding on probing, colour change, marked swelling with or without ulceration.

CALCULATION:

Each of the four gingival units (M and P) is scored from 0 to 5 to obtain the SBI of the area. The scores for the four units are added and further divided by four to obtain the SBI for desired tooth.

PROBING POCKET DEPTH (PPD): (Fig. 13a, 13b, 14a, 14b)

The Probing Pocket Depths were measured from the crest of the marginal gingiva to the base of the pocket using William's periodontal probe. The probe was inserted parallel to the vertical axis of the tooth and 'walked' circumferentially around each surface of each tooth to detect the areas of deepest penetration.

CLINICAL ATTACHMENT LEVEL (CAL):

The distance from Cemento-enamel Junction to the bottom of the pocket was measured using William's periodontal probe.

BIOCHEMICAL ANALYSIS FOR DETECTION OF ALKALINE PHOSPHATASE LEVELS IN GCF:

Clinical procedure for collection of GCF:

1. The subjects were seated comfortably in the dental chair in an upright position.
2. Scaling and root planing was done for the removal of plaque and calculus.
3. Two sites in two contralateral quadrants were selected with a periodontal pocket depth of > 4 mm for the collection of GCF sample by using coin-

toss method at baseline and at 4 weeks after completion of the desired periodontal treatment.

4. The sites were then dried with a gentle stream of compressed air. Absorbent cotton rolls and a saliva ejector were used to maintain isolation during the GCF collection.
5. The calibrated micro-capillary tube (0 – 5 μL) was placed extra-crevicularly and a standardized volume of 5 μL was collected. (Fig. 10) The micro-capillary tubes which were contaminated with blood or inadequate volume of fluid were discarded.
6. After collection of GCF from both the sites GCF was transferred in sterilized micro-centrifuge tubes (Eppendorf tubes) containing 45 μl of normal saline. (Fig. 11)
7. The samples were stored at -80 degree Celsius until they were assayed for Alkaline phosphatase. The samples were transported immediately to the Department of free Biochemistry, KAHER's K.L.E. Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi where they were assayed for levels of Alkaline Phosphatase with the use of Biochemical kit (Erba Mannheim Chemicals)

Determination of alkaline phosphatase levels in GCF:

Alkaline Phosphatase levels were determined using Bio-chemical kit (Erba Mannheim Chemicals) at the Department of Free Biochemistry, KAHER's K.L.E. Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi by the technician who was blinded for the patient's characteristics as well as study design. (Fig. 15a).

The values obtained after analysis on the biochemical analyzer (Fig. 15b) were interpreted in U/L.

RECORDING OF PARAMETERS AT 4 WEEKS POST-TREATMENT: (Fig. 12b, 13b)

Patients were re-assessed at 4 weeks post-treatment for Plaque Index, Sulcus Bleeding Index, Probing Pocket Depth (Fig. 12b, 13b), Clinical Attachment Levels and Alkaline Phosphatase levels in GCF samples.

STATISTICAL ANALYSIS:

The means and standard deviation were calculated for the clinical and biochemical parameters for both test and control groups at baseline as well as at 4-week post-operative follow up.

Intra-group comparison for clinical and biochemical parameters from baseline to 4 weeks was performed using paired student-t test, while intra-group comparison between test and control groups was done using Z test.

Probability value (p-value) of <0.05 was considered to be statistically significant whereas $p<0.001$ was considered statistically highly significant.

The data was processed for statistical analysis using Statistical analysis software SPSS 20.0.

PHOTOGRAPHS

FIG 1:

Ashwagandha leaves Ginger rhizome

FIG 2: Ethanolic extracts of Ashwagandha leaves and Ginger rhizome

FIG 3: Resazurin Dye Method using 96 well plates for determination of MIC

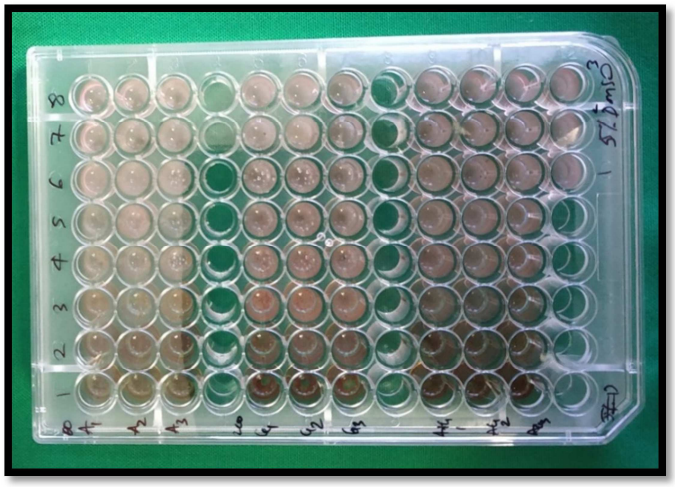


FIG 4a: Results of MBC Ashwagandha (MBC – 6.8 mg)

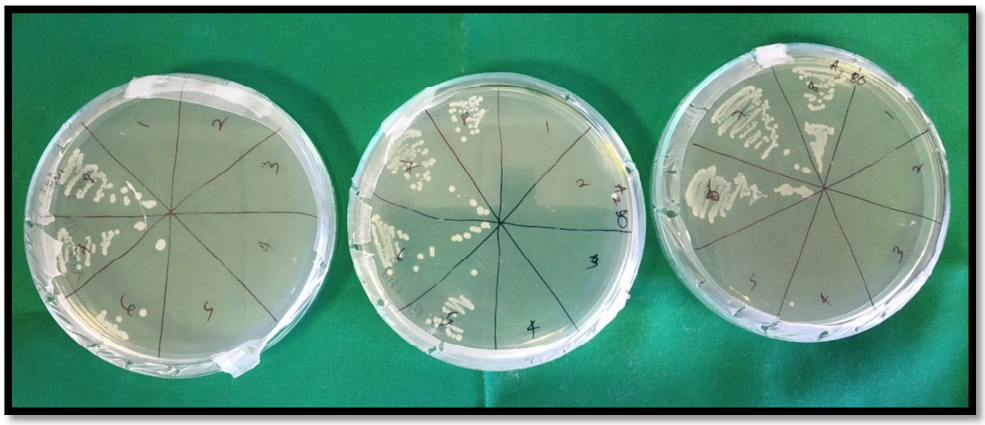


FIG 4b: Results of MBC Ginger (MBC - 130mg)

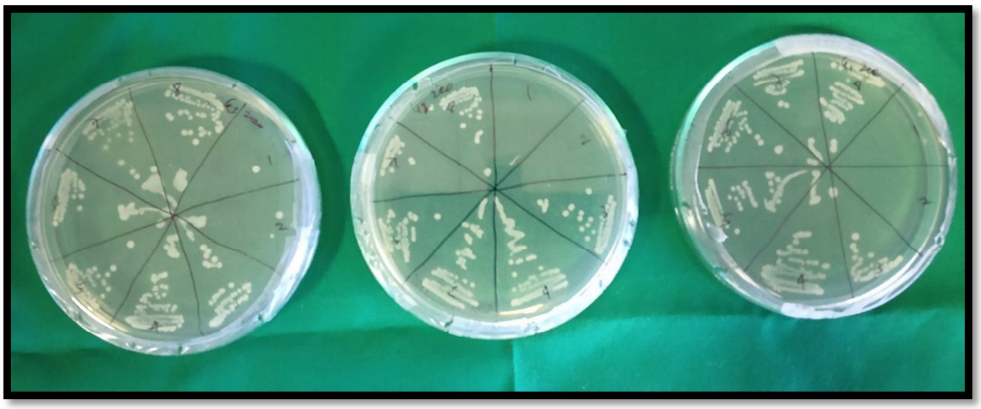


FIG 4c: Results of MBC Ashwagandha and Ginger in combination (MBC – 35mg)

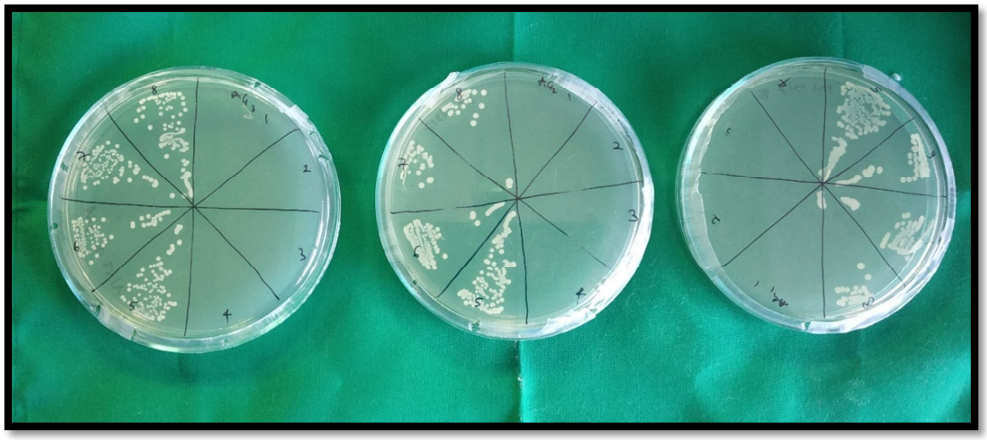


FIG 5: Armamentarium for Polyherbal preparation
Magnetic stirrer Weighing scale

FIG 6: Sterilization of gel using UV radiation

Polyherbal gel

FIG 7: Armamentarium used for the clinical study

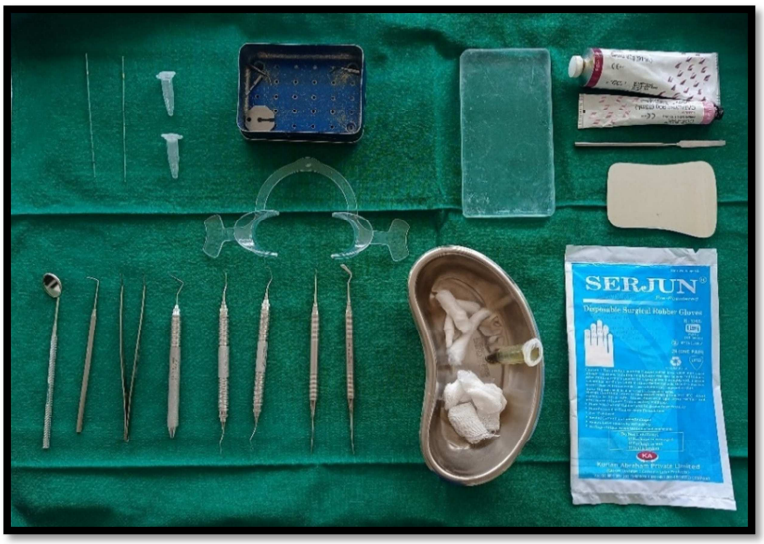


FIG 8a: Before Scaling and Root Planing

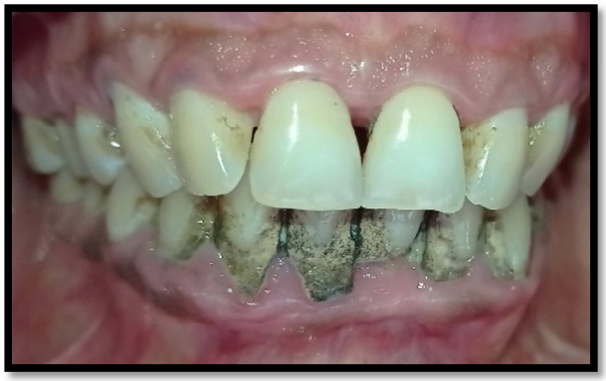


FIG 8b: After Scaling and Root Planing

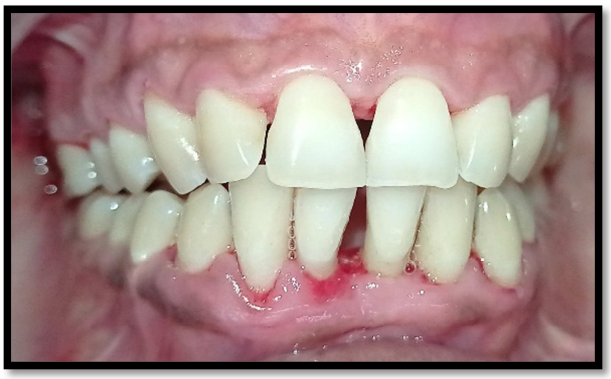


FIG 9: GCF collection (5µL) from target site and its transfer into Eppendorf tube containing normal saline (45 µL)

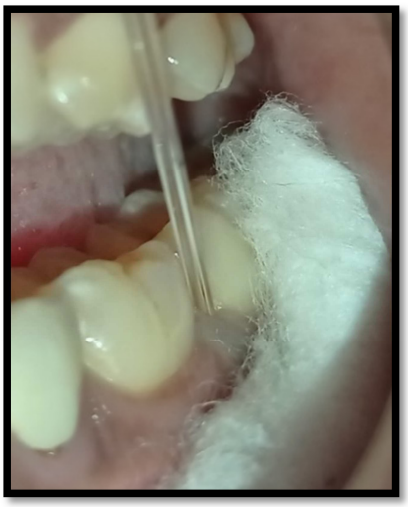


FIG 10: GCF collected from test and control group from target site in an Eppendorf tube

FIG 11: Gel placement at the target site in test group

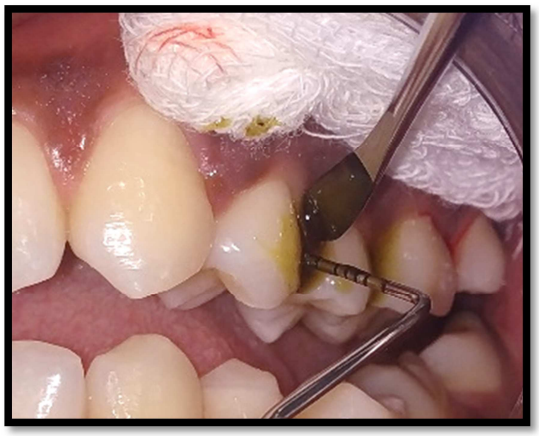


FIG 12: Periodontal pack placement at target site in test group

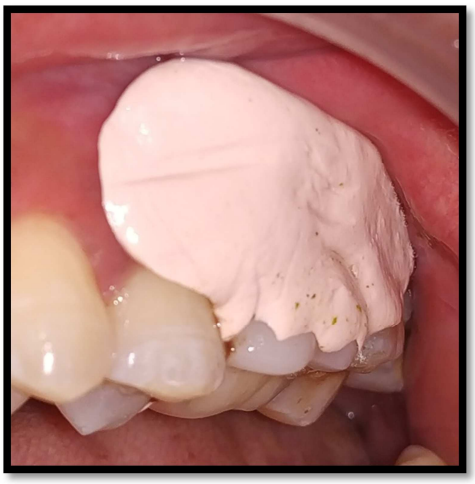
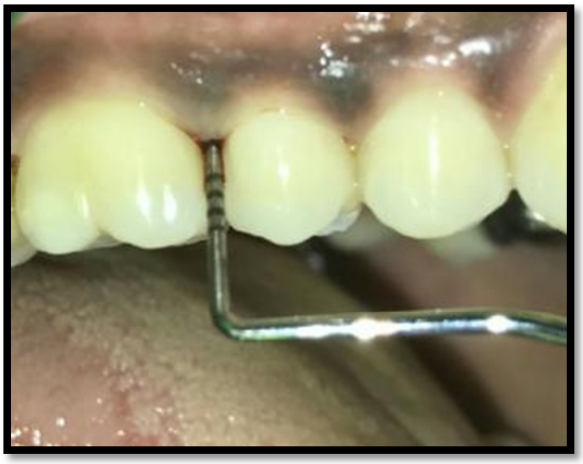


FIG 13: The measurement of PPD at target site in test group

13a: At baseline 13b: At 4 weeks post-treatment



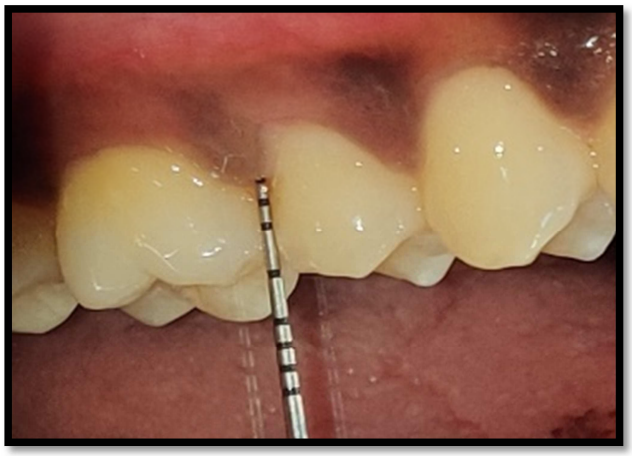
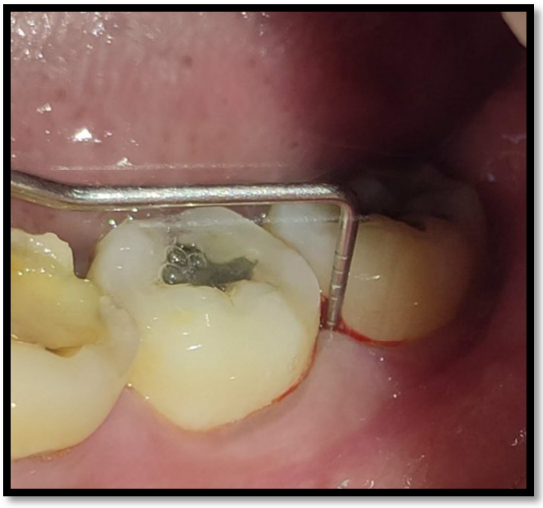


FIG 14: The measurement of PPD at target site in control group

14a: At baseline 14b: At 4 weeks post-treatment



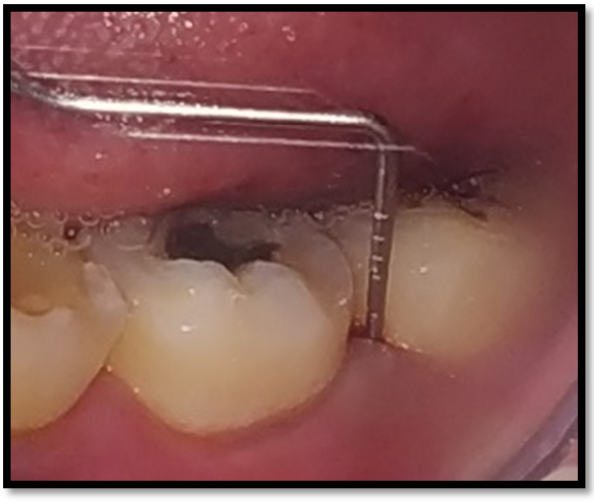


FIG 15a: Reagents of the biochemical kit for estimation of ALP levels in GCF

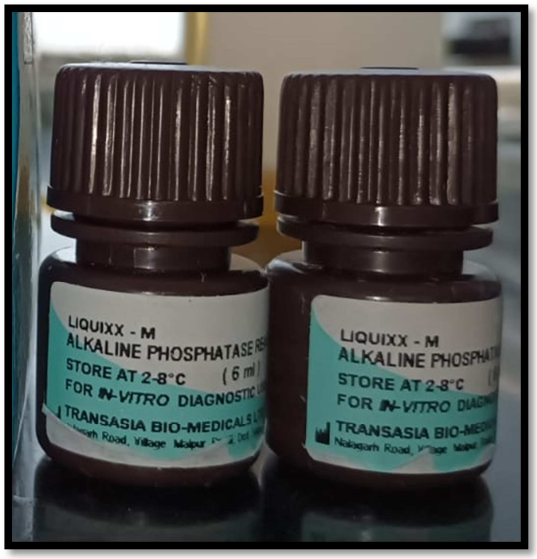


FIG 15b: Biochemistry Analyzer for estimation of ALP levels in GCF

RESULTS AND OBSERVATIONS

Table 1: Descriptive statistics of the study participants

SEX			AGE	
Gender	Frequency	Percent	MEAN AGE (In years)	Std. Deviation
Female	10	43.5	32.2	8.32399
Male	13	56.5	33.6	9.16095
Total	23	100.0	33.0	8.63923

Observations of Table 1 (Graph 1):

The total number of participants included in the study were 23. Out of them 10 were females (43.5%), whereas 13 were males (56.5%).

The mean age of females included in the study was 32.2 years and that of males was 33.6 years.

Mean age of all the participants both male and female was 33 years. (Graph 1)

Table 2: Intra-group and Inter-group distribution and comparison of Pocket Probing Depth scores at baseline and at 4 weeks between Test and Control groups

PPD (in mm)				
	TEST		CONTROL	
Group	BASELINE	4 WEEKS	BASELINE	4 WEEKS
Mean	5.18	2.99	4.98	3.35
Std. Deviation	0.392	0.612	0.553	0.776
Mean Difference	2.187		1.626	
t test[‡]	14.433		8.18	
p-values*	0.000		0.000	

*p value < 0.001 is statistically highly significant.

[‡] Test applied – paired t-test for intra-group comparison

Z test in between TEST and CONTROL Group for intergroup comparison of Pocket Probing Depth; Z = 10.744; p – values < 0.001

Observations of Table 2 (Graph 2):

The mean PPD scores for Test group at baseline and at 4 weeks were 5.18 ± 0.39 mm and 2.99 ± 0.61 mm respectively. In Control group, the mean PPD score at baseline was 4.98 ± 0.55 mm whereas, for 4 weeks it was 3.35 ± 0.78 mm.

Mean difference from baseline to 4 weeks in Test group was 2.187 mm while that for Control group it was 1.626 mm.

On intra-group comparison, the improvement from baseline to 4 weeks was found statistically highly significant for both the test ($p<0.001$) and control ($p<0.001$) groups.

Inter-group comparison between test and control group using Z test showed that there was statistically highly significant ($p<0.001$) improvement in the test group than that of control group at the end of 4 weeks.

Table 3: Intra-group and Inter-group distribution and comparison of Clinical Attachment Level scores at baseline and at 4 weeks between Test and Control groups

CAL (in mm)				
	TEST		CONTROL	
Group	BASELINE	4 WEEKS	BASELINE	4 WEEKS
Mean	3.35	1.2	3.2	1.67
Std. Deviation	0.51	0.517	0.619	0.902
Mean Difference	2.152		1.535	
t test[‡]	14.222		6.725	
p-values*	0.000		0.000	

*p value < 0.001 is statistically highly significant.

[‡] Test applied – paired t-test for intra-group comparison

Z test in between TEST and CONTROL Group for intergroup comparison of Clinical Attachment Level; Z = 10.82036; p - values < 0.001

Observations of table 3 (Graph 2):

In Test group, the CAL scores at baseline and 4 weeks were 3.35 ± 0.51 mm and 1.2 ± 0.51 mm while in Control group they were 3.2 ± 0.61 mm and 1.67 ± 0.90 mm respectively.

On intra-group comparison, mean difference from baseline to 4 weeks in Control group was 1.53 mm whereas in Test group it was 2.15 mm. The results of t test showed that this mean difference was statistically highly significant in both the groups at the end of 4 weeks ($p<0.001$).

Inter-group comparison using Z test at the end of 4 weeks showed that test group showed statistically highly significant improvement ($p<0.001$) than that of control group.

Table 4: Intra-group and Inter-group distribution and comparison of Sulcus Bleeding Index scores at baseline and at 4 weeks between Test and Control groups

SBI				
	TEST		CONTROL	
Group	BASELINE	4 WEEKS	BASELINE	4 WEEKS
Mean	2.02	0.78	1.97	0.91
Std. Deviation	0.767	0.717	0.839	0.596
Mean Difference	1.246		1.052	
t test[‡]	5.69		4.901	
p-values*	0.000		0.000	

*p value < 0.001 is statistically highly significant.

[‡] Test applied – paired t-test for intra-group comparison

Z Test in between TEST and CONTROL Group for intergroup comparison of Sulcus Bleeding Index; Z = 3.031604; p - values < 0.001

Observations of table 4 (Graph 3):

The mean SBI Scores at baseline and 4 weeks in the control group was 1.97 ± 0.83 and 0.91 ± 0.59 respectively whereas in the test group it was 2.02 ± 0.76 at baseline while 0.78 ± 0.71 at 4 weeks.

The mean difference in the control group from baseline to 4 weeks was 1.05 however for test group it was 1.24. It was observed that, there was statistically highly

significantly improvement in the test group than control group ($p<0.001$) on performing Z test.

Both the test and control group showed statistically highly significant improvement from baseline to 4 weeks on performing t test ($p<0.001$)

Table 5: Intra-group and Inter-group distribution and comparison of Plaque Index scores at baseline and at 4 weeks between Test and Control groups

PI				
	TEST		CONTROL	
Group	BASELINE	4 WEEKS	BASELINE	4 WEEKS
Mean	1.81	0.8	1.69	1.07
Std. Deviation	0.68	0.488	0.763	0.546
Mean Difference	1.015		0.626	
t test[*]	5.82		3.2	
p-values*	0.000		0.003	

*p value < 0.001 is statistically highly significant.

[*] Test applied – paired t-test for intra-group comparison

Z Test in between TEST and CONTROL Group for intergroup comparison of Plaque Index;

Z = 7.11804; p - values < 0.001

Observations of table 5 (Graph 3):

For the test group, the mean PI scores at baseline and 4 weeks were 1.81 ± 0.68 and 0.8 ± 0.48 respectively. However, for the control group they were 1.69 ± 0.76 and 1.07 ± 0.54 respectively.

Mean difference for PI between baseline to 4 weeks in the control group was 0.62 while for test group it was 1.01. t test showed statistically highly significant improvement at the end of 4 weeks in test group ($p<0.001$) whereas it was significant in control group. ($p<0.05$)

Inter group comparison between test and control group using Z test showed statistically highly significant improvement in test group than that of control group ($p<0.001$)

Table 6: Intra-group and Inter-group distribution and comparison of Alkaline Phosphatase Levels in Gingival Crevicular Fluid at baseline and at 4 weeks between Test and Control groups

GCF ALP (U/L)				
	TEST		CONTROL	
Group	BASELINE	4 WEEKS	BASELINE	4 WEEKS
Mean	583.16	198.93	510.7	267.58
Std. Deviation	445.61	123.001	337.781	212.039
Mean Difference	384.23		243.122	
t test[*]	3.986		2.924	
p-values*	0.000		0.005	

*p value < 0.001 is statistically highly significant.

[*] Test applied – paired t-test for intra-group comparison

Z Test in between TEST and CONTROL Group for intergroup comparison of Alkaline Phosphatase levels in Gingival Crevicular Fluid; Z = 5.3157; p - value < 0.001

Observations of table 6 (Graph 4):

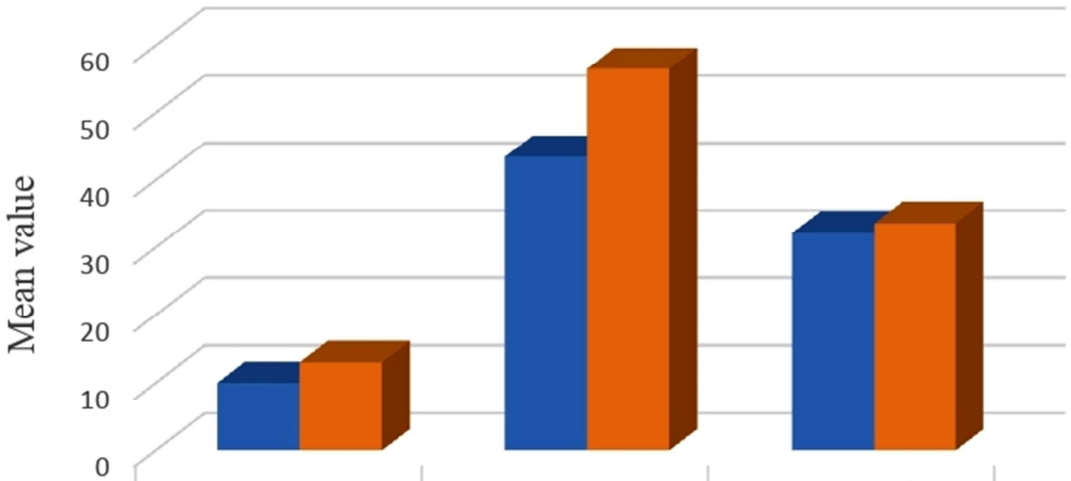
In the test group, the mean ALP levels in GCF at baseline were 583.16 ± 445.61 U/L whereas at 4 weeks they were 198.93 ± 123.00 U/L. This intra-group difference showed statistically highly significant improvement ($p<0.001$) at the end of 4 weeks in the test group.

In the control group, the mean ALP levels in GCF at baseline were 510.7 ± 337.78 U/L and at 4 weeks they were 267.58 ± 212.03 U/L, which was found statistically significant ($p<0.05$) upon performing t test.

In the test group, mean difference from baseline to 4 weeks was 384.23 U/L while in the control group it was 243.122 U/L.

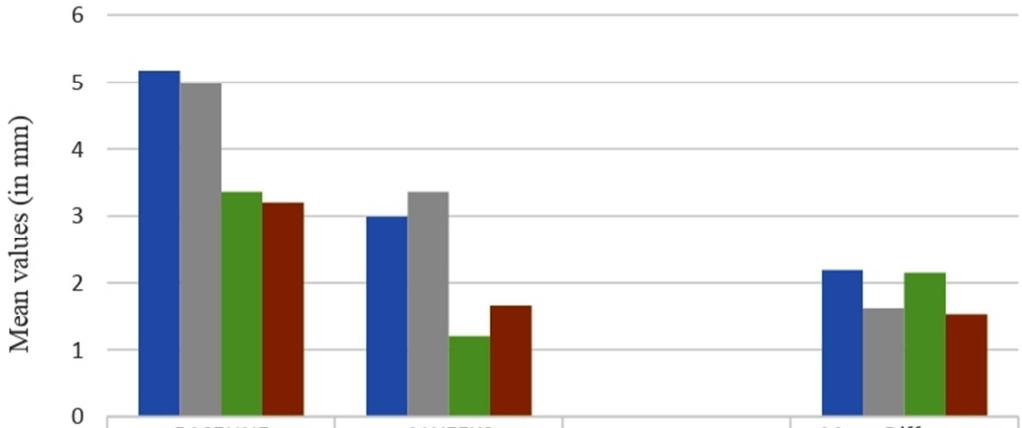
Inter-group comparison between test and control groups by Z test at 4 weeks showed statistically highly significant improvement in the test group ($p<0.001$) than that of control group.

Graph 1: Demographic data of patients



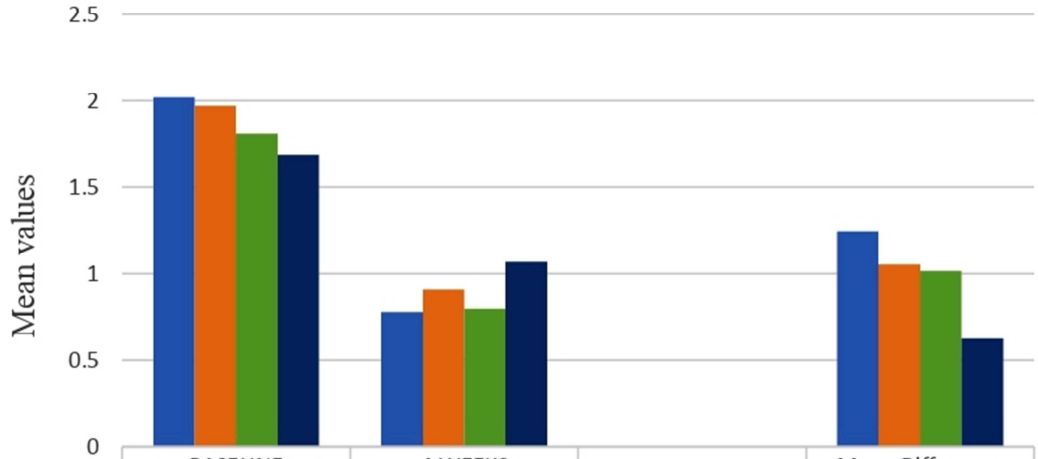
	Frequency	Percent	MEAN AGE (in years)
female	10	43.5	32.2
male	13	56.5	33.6

Graph 2: Inter and Intra- group comparison of Test and Control group with respect to Pocket Probing Depth and Clinical Attachment Level at Baseline and at 4 weeks



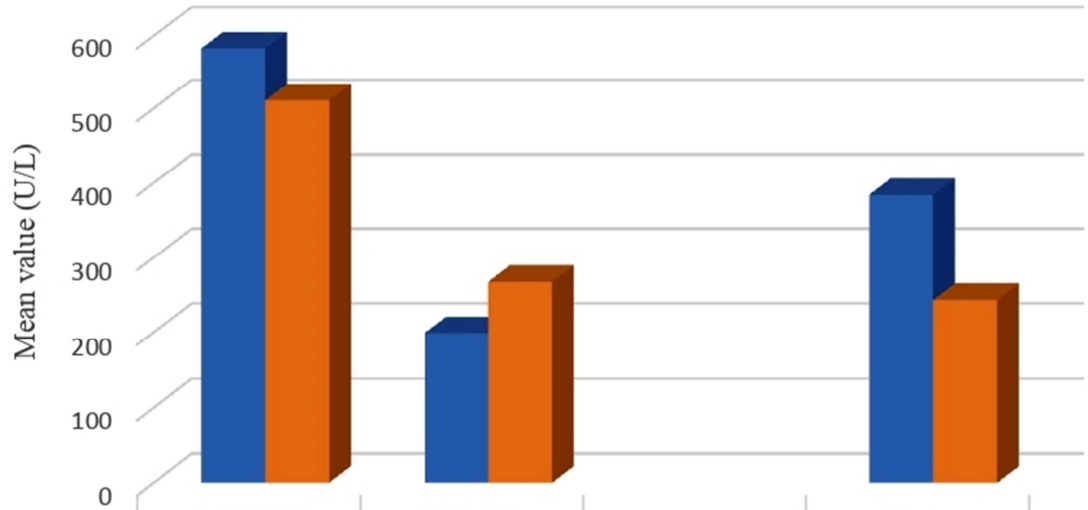
	BASELINE	4 WEEKS		Mean Difference
PPD TEST	5.18	2.99		2.187
PPD CONTROL	4.98	3.35		1.626
CAL TEST	3.35	1.2		2.152
CAL CONTROL	3.2	1.67		1.535

Graph 3: Inter and Intra- group comparison of Test and Control group with respect to Sulcus Bleeding Index and Plaque Index at Baseline and at 4 weeks



	BASELINE	4 WEEKS		Mean Difference
SBI TEST	2.02	0.78		1.246
SBI CONTROL	1.97	0.91		1.052
PI TEST	1.81	0.8		1.015
PI CONTROL	1.69	1.07		0.626

Graph 4: Inter and Intra- group comparison of Test and Control group with respect to Alkaline Phosphatase levels in Gingival Crevicular Fluid at Baseline and at 4 weeks



	BASELINE	4 WEEKS		Mean Difference
TEST	583.16	198.93		384.23
CONTROL	510.7	267.58		243.122

DISCUSSION

Periodontitis is a chronic inflammatory disease characterized by gingival inflammation initiated by microbial plaque eventually leading to the destruction of periodontal ligament further resulting in alveolar bone resorption.[20] Periodontal therapy most importantly focuses on Scaling and root planing (SRP) for the removal of biofilm, primarily to reduce inflammation, although mechanical instrumentation alone sometimes fails to reach the deposits in deep inaccessible areas of the periodontal pockets.[5] Therefore, with the growing interest and increasing knowledge about the medicinal value of natural herbal products, various formulations have been prepared to overcome the side effects of synthetic chemotherapeutic agents available like doxycycline, minocycline, metronidazole, chlorhexidine when used either systemically or sub-gingivally as a Local drug delivery (LDD) agent. Numerous periodontal diagnostic procedures are currently available so as to offer information to the practitioner regarding the existing periodontal disease type, its location and severity which can be served as a basis for the treatment planning with further evaluation, maintenance and disease monitoring along the duration of the treatment.[20]

Therefore, the present study was aimed to evaluate and compare the efficacy of a polyherbal gel containing Ashwagandha and Ginger as a Local Drug Delivery Agent, on the clinical and biochemical parameters in patients diagnosed with chronic periodontitis as an adjunct to scaling and root planing.

Ashwagandha was selected based on its anti-inflammatory, immunomodulatory, anti-bacterial, anti-tumorigenic and antioxidant properties. Ashwagandha leaves were selected for the extract preparation as studies showed that Withaferin A (WA), the biologically active steroid present in the leaf extract shows

chief anti-inflammatory action similar to commercially available NSAIDs.[12] Whereas Ginger was chosen because of its anti-microbial, anti-oxidant, immunostimulant, cardio-protective and anti-cancer properties. Pain-relieving and anti-inflammatory properties of ginger were primarily attributed to the presence of Gingerol, an active constituent, in conditions like arthritis and osteoarthritis.[14]

Oral administration of composite extract containing *Withania somnifera*, *Ocimum sanctum* and *Zingiber officinale* (Oxitard™) was done in male Wistar strain rats to assess its anti-oxidative potency in comparison with a standard, potent antioxidant – vitamin E. As a result, they found that there was a significant increase in the level of antioxidants (serum ProCT) as well as clinical parameters like GI, PPD and CAL in Oxitard receiving group with equivalent potency to vitamin E. (Sekhar Misra D et al., 2005)[26]

To our knowledge, until now, there have been no studies conducted using a combination of Ashwagandha and Ginger as LDD agent in dentistry, though there are several studies evaluating their anti-cancer[39] and health promoting activity when administered orally[40,41] either alone or in combinations with other herbs.

The pilot study was conducted against *Staphylococcus aureus* a gram-positive, facultative aerobe to ascertain MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values of Ashwagandha, Ginger and a combination of both. For Ashwagandha (*Withania somnifera*) mean MIC value was found to be 6.60mg/ml whereas the mean MBC value was 5mg/ml. For Ginger, it was 130mg/ml and 100mg/ml respectively whereas when used in combination, MIC and MBC were found to be 35mg/ml. (Annexure VIII, Fig. 3,4a, 4b, 4c) Based on these results, Ashwagandha and Ginger were incorporated in the polyherbal gel formulation

for local drug delivery with their concentration around 100 times higher than their MIC reported. This was mainly because many biofilm experiments indicated that the necessary minimum inhibitory concentrations of antimicrobial agents should be at least 50 times or even 210,000 times higher than that for bacteria growing under planktonic conditions to demonstrate their desired effects.[66] Ethanolic extracts were preferred as highest anti-oxidant properties[55,67] of Ashwagandha and Ginger both were found in Ethanolic extracts (lowest IC_{50} values) followed by water-ethanol and least in aqueous extracts.

The present study was a randomized, controlled, double-blind, split-mouth clinical trial. The split-mouth study design was incorporated as it eliminates interpatient systemic differences which can have an effect on outcomes of the periodontal therapy,[68] helps maintain the same study environment for both Test and control groups avoiding performance bias.

The study included total of 23 participants with a mean age of 33 years (Table 1, Graph 1), all diagnosed with Chronic periodontitis who were reported to the Department of Periodontics, KAHER's KLE V. K. Institute of Dental Sciences, Belagavi.

Reasons for mean age less than 50 years could be due to unwillingness of patients to participate in the study (not willing to give consent), their incapacity to come for follow up at the end of 4 weeks (patient lost to follow up due to any reason) and patients with systemic problems including diabetes, hypertension, hyper or hypothyroidism, history of cardiac surgery, leading to their exclusion from the study.

In each patient, 2 sites in contra-lateral quadrants were selected (to avoid "carry-across" or "spillover" effects[68]) fulfilling the eligibility criteria mainly to avoid cross-contamination of the control site with the test group.

The sites were randomly divided into Test and Control groups using the coin-toss method (to avoid selection bias). Test group (n=23) consisted of sites where polyherbal gel containing Ashwagandha and Ginger was applied after complete removal of plaque and calculus post-SRP. However, in the Control group (n=23) Scaling and Root Planing was performed alone as a periodontal therapy.

The clinical parameters recorded at baseline and at 4 weeks post-treatment were Plaque Index (PI), Sulcus Bleeding Index (SBI), Pocket Probing Depth (PPD) and Clinical Attachment Levels (CAL) while as a biochemical parameter, levels of Alkaline Phosphatase (ALP) in the Gingival Crevicular Fluid were measured.

Follow up period was chosen to be of 4 weeks as it was observed by Goldman that after 14 to 16 days of healing following SRP, there is a considerable decrease in the inflammatory cell infiltration along with a virtual absence of chronic cellular infiltrate up to 6 weeks. Also, there is formation of collagen and improved organization of tissues gradually over a 3–4-weeks period. Therefore, re-evaluation of probing after scaling and root planing was performed after 4 weeks, as at this time soft tissues reached complete maturation and the patient got sufficient time to be habitual with oral hygiene techniques for future maintenance.[69]

The Plaque Index (PI) is one of the most extensively used indices, as it is easy to perform, requires less time to access and shows improvement in accordance with periodontal treatment progression. Therefore, it can give an insight into an

individual's level of oral hygiene maintenance. It also has the ability to distinguish clearly between the severity and location of the soft debris aggregates intraorally. The plaque index also shows good feasibility, reliability and consistency in the evaluation of various anti-plaque procedures in due course of the treatment.[63]

Therefore, in the present study, we incorporated PI as one of the outcome variables. Intragroup comparison from baseline (1.81) to 4 weeks (0.8) showed statistically highly significant improvement in the test group ($p<0.001$) whereas in the control group (at baseline – 1.69, at 4 weeks – 1.07) it was statistically significant ($p<0.01$). Intergroup comparison between test (1.01) and control (0.62) group demonstrated statistically highly significant ($p<0.001$) improvement in the test group (Table 5, Graph 3) which could be credited to the synergistic effect of properties of ashwagandha and ginger containing polyherbal gel showing an enhanced effect on SRP due to their anti-bacterial[11,12] and broad-spectrum biofilm inhibiting property against periodontopathogens.[14,16,51] Whereas, the improvement in the control group could be due to scaling and root planing which created an environment, less favorable for the formation and accumulation of microbes and biofilm. Additionally, oral hygiene instructions might also help in the better reduction of plaque scores.

This was in accordance with the findings of the study where ginger showed biofilm inhibitory action against *Pseudomonas aeruginosa* PA14 when static biofilm assay was used. This inhibition of biofilm formation was through reduction of cellular c-di-GMP along with alteration of its phenotype without causing any alteration in Quorum sensing (QS). (Han-Shin Kim and Hee-Deung Park, 2013)[51] However, Sana'a MA and Ahmed MA in 2017 showed that concentrations of alcoholic extract of Ginger had more antibacterial activity as inhibition zones were having higher mean

values for *Aggregatibacter actinomycetemcomitans* (A. a.) than the concentrations of aqueous extract which was might be due to the presence of 10-gingerol as one of the active constituents of Ginger extract.[48] Similarly, the protective activity of WA (active constituent of Ashwagandha leaf) against various bacteria could be related to activation of macrophage function due to associated increase in phagocytosis along with the intracellular killing of peritoneal macrophages as described by Dhuley 1998.[29]

Sulcus Bleeding Index (SBI) in particular is useful for early detection of minute inflammatory changes at the base of the periodontal pocket which is usually considered as an inaccessible area for direct visual examination. As soon as the disease process starts, bleeding is considered as the first objective sign to occur, giving an idea about the disease progression. SBI provides an easily reproducible measurement of the status of the gingival sulcus both pre-and post-treatment.[70]

In the existing study, intragroup comparison from baseline to 4 weeks follow up showed statistically highly significant ($p<0.001$) improvement in both test (baseline – 2.02, 4 weeks – 0.78) and control (baseline – 1.97, 4 weeks – 0.91) groups. (Table 4, Graph 3). Whereas, inter-group comparison between test (1.24) and control (1.05) groups showed statistically highly significant ($p<0.001$) improvement in the test group which might be due to anti-inflammatory[12,13,15,71] and anti-bacterial[16,32,33] properties of the polyherbal gel containing Ashwagandha and Ginger due to inhibition of NFκB activation[12] and PGE2 production[13] by Ashwagandha and non-selective cyclo-oxygenase (COX) inhibition by Ginger[15] showing improvement in the SBI scores, decreasing the bleeding further. However, in the control group, this

can be due to a reduction in inflammation after SRP along with patient education and motivation for maintenance of oral hygiene.

These findings might support another study where inflammation was induced in the zebrafish using stainless steel implants to evaluate the Anti-inflammatory activity of Ashwagandha when injected intramuscularly. Results showed significant inhibition of TNF-α, owing to reduction in inflammation might be due to the presence of flavonoids and phenolic acids in the Ashwagandha extract. (Sivamani S et al. 2014)[11] Similar results were observed in a study in which silver nanoparticles (AgNPs) were biosynthesized using fungi obtained from Ashwagandha leaves to determine its antibacterial efficacy against *Porphyromonas gingivalis* using agar well diffusion method in comparison with CHX and ampicillin, results showed the efficacy of AgNPs was equivalent to both CHX and ampicillin showing prominent anti-bacterial activity. (Halkai KR 2017)[32]

In another study, antimicrobial activity of [10]-gingerol was found higher than that of [12]-gingerol, when studied against *Porphyromonas* and *Prevotella* species using Ginger extract. (Miri Park et al., 2008)[16] Likewise, Javid AZ et al. (2019) have evaluated results of supplementation with 2 gm Ginger tablets (4 tablets of 500 mg each) orally for 8 weeks in patients with Type 2 Diabetes Mellitus (DM) and chronic periodontitis together along with SRP and found effective improvement in inflammation, oxidative stress and periodontal status.[52] All of these findings support the action of both ashwagandha and ginger against periodontopathogens.

In the current study, reduction in the pocket probing depth (PPD) was statistically significant ($p<0.001$) in both test (baseline – 5.18mm, 4 weeks – 2.99mm) and control (baseline – 4.98mm, 4 weeks – 3.35mm) groups from baseline to 4 weeks

follow up showing statistically significant ($p<0.001$) greater reduction in the test (2.18mm) than control (1.62mm) group. (Table 2, Graph 2). This could be ascribed to the reduction in the puffiness/ swelling of marginal gingiva due to a decrease in the gingival inflammation owing to the anti-inflammatory property of the gel constituents in the test group, whereas in the control group this could be due to a reduction in the inflammation caused because of removal of local deposits and its maintenance by the patient promoting healing of the periodontal pocket lining.

A higher reduction of PPD in the test group might be due to the anti-bacterial property of the Ashwagandha and ginger analogous to the results of in-vitro studies done by Yahya et al. (2013) where they found that the ethanolic extract of Ginger inhibited *Pseudomonas aeruginosa* biofilm formation.[33] Similarly, in another study authors evaluated antibacterial (by microdilution method) and anti-biofilm activity (by crystal violet assay) of ethanolic extract from the rhizome of Ginger on *Pseudomonas aeruginosa* biofilm showing moderate anti-biofilm activity of ginger. (Nikolić M et al. 2014)[50]

Nevertheless, Singariya P et al. (2012)[34] confirmed the broad-spectrum antibacterial activity of alcoholic extract of WS against *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Raoultella planticola, Enterobacter aerogens,* while Thanwar Mayuri evaluated anti-bacterial properties of Ashwagandha using disc diffusion method on *Pseudomonas aeruginosa*.[35] Due to minimal bacterial accumulation, there was a reduction in resultant immune response leading to a further decrease in the inflammation and pocket formation resulting in a decrease in the PPD.

Along with PI, SBI and PPD, CAL also showed statistically significantly greater improvement in the test (baseline – 3.35mm, 4 weeks – 1.2mm; mean difference – 1.53) group than control (baseline – 3.2mm, 4 weeks – 1.67mm; mean difference – 1.53) group ($p<0.001$) at the end of 4 weeks when compared from baseline although both the groups showed enhancement in CAL on the intra-group comparison (Table 3, Graph 2). In the test group, this could be mainly attributed to wound healing properties of Ashwagandha[21] and ginger[56] which will enhance the healing of epithelium leading to a decrease in attachment loss and pocket probing depth, while in the control group, it could be due to epithelium and connective tissue healing followed by SRP.

The Anti-inflammatory activity and its effect on the number of neutrophils, fibroblast cells and epithelialization in incision wounds were proven in an animal study done on hairless rats where the topical application of 10% curcumin, 3% ginger and in combination showed rapid healing in the skin of rats. (Bhagavathula N et al. 2009)[57] This healing was attributed to the activity of gingerol as it inhibits the COX activity followed by a decrease in prostaglandin (PG) synthesis which causes a decrease in inflammatory mediators further reducing the number of neutrophils thereby reducing inflammation. (Rahayu KI et al. 2020)[56]

Min K. et al in 2011[71] described the anti-inflammatory property of Ashwagandha in murine microglial cells where they showed inhibition of COX-2 mRNA, protein expression and PGE2 production. Ashwagandha has shown accelerated wound healing property when used in the treatment of toe gangrene. (Sheoran S et al. 2020)[39] Flynn and Rafferty proved that some of the constituents of ginger such as [6]-gingerol, shagaol, gingerdione and dihydroparadol act as functional

dual inhibitors of arachidonic acid metabolism. This probably explains why high doses of Ginger extract do not show side effects often observed with traditional non-selective COX inhibitors. Pallavi Menon et al. in 2020[47] reported a reduction in pain scores (VAS scores) and Modified Gingival Index scores on oral administration of Ginger capsules in patients with chronic generalized periodontitis undergoing open flap debridement.[47]

As a widely accepted notion, the presence of ALP in the GCF is indicative of inflammation or destruction of the periodontal tissues and is considered to be positively correlated with the severity of periodontal disease.[20] In our study, ALP levels were measured at baseline and 4 weeks post-treatment in the test group (baseline – 583.16 U/L, 4 weeks – 198.93 U/L) and control group (baseline – 510.7 U/L, 4 weeks – 267.58 U/L). They showed a statistically highly significant ($p<0.001$) decrease in the GCF ALP levels in the test group (384.23 U/L) than the control group. This may be credited to the anti-inflammatory and anti-oxidant property of both Ashwagandha and Ginger, where they inhibit PG synthesis via non-selective inhibition of COX[13,15] resulting in reduction in oxidative stress.[11,26,38] The reduction in the GCF ALP values in the control group (243.12 U/L) could be due to the reduction of the inflammation due to the complete removal of the local factors during SRP. (Table 6, Graph 4)

Our results were in agreement with the studies conducted on animal models, where it has been seen that WS extract showed increase in free radical scavenging enzymes like superoxide dismutase (SOD), catalase (CAT) and general peroxidases (GPX) activity (in rat brain) proving the anti-oxidant capability of Ashwagandha.[29] V Khedgikar et al in 2013[40] conducted a study where WA was administered orally in

osteopenic ovariectomized mice. They observed WA acting as a proteasomal inhibitor (PI*) showed its immunomodulatory properties.[40] On administration of polyherbal anti-oxidant capsules (Oxitard™) containing ashwagandha in the smokers having chronic periodontitis, Sravya MN, et al. in 2019[38] discerned statistically significant improvement in serum procalcitonin (ProCT) levels in the test group when compared with the control group involving SRP alone. Similarly, in-vitro studies have shown, ginger when evaluated, exhibited anti-oxidant properties. [44,55] Hence, the anti-oxidant property of Ashwagandha and ginger could be the reason for the reduction in oxidative stress and periodontal disease progression further.

Ashwagandha and ginger act as an anti-oxidant and immunomodulator, modulating the activity of free radical scavenging enzymes to reduce inflammation[12,14] which will further slow down disease progression along with inhibition of expression of various chemokines, TNF-α, IL-1β, NF-κB .[15]

Thus, the properties of both Ashwagandha and Ginger can prove beneficial in the treatment of inflammatory diseases like chronic periodontitis, contributing to the reduction in various clinical and biochemical outcome variables depicting a decline in the disease progression.

Therefore, within its limits, our study suggested the successful use of a polyherbal gel containing Ashwagandha and ginger as an adjunct to scaling and root planing in improving clinical and biochemical parameters when compared to conventional SRP alone.

SUMMARY AND CONCLUSION

The present study was conducted with the aim of evaluating and comparing the efficacy of the administration of polyherbal gel containing Ashwagandha and Ginger as an adjunct to scaling and root planning in patients with chronic periodontitis. The study was conducted in the Outpatient Department, Department of Periodontics, KAHER's KLE V K Institute of Dental Sciences, Belagavi.

A total of 23 patients diagnosed with chronic periodontitis participated in this randomized controlled trial. Considering the split-mouth design, in each patient, two sites in the contralateral quadrants following eligibility criteria were selected. The sites were randomly divided into Test and Control groups. Both the groups consisted of 23 patients each, wherein in the Test group participants, Polyherbal gel containing Ashwagandha and ginger was administered as a Local drug delivery agent as an adjunct to scaling and root planing. In Control group Scaling and Root planning was performed. The outcome variables measured were clinical parameters including Plaque Index (PI), Sulcus Bleeding Index (SBI), Pocket Probing Depth (PPD) and Clinical Attachment Levels (CAL) along with biochemical parameter – estimation of Alkaline Phosphatase levels in Gingival Crevicular Fluid (GCF ALP). The parameters were assessed at baseline as well as 4 weeks post-operatively.

Intra-group comparison from baseline to 4 weeks showed statistically significant improvement in all the outcome variables in both Test and Control groups. Whereas, on inter-group comparison between Test and Control groups, statistically highly significant improvement was seen in the Test group (SRP + polyherbal gel) when outcome variables such as PI, SBI, PPD, CAL along with GCF ALP were measured at the end of 4 weeks.

Within the limitations of the study, the following conclusions were put together:

1. On Intra-group comparison at 4 weeks from baseline, the Control group showed statistically significant improvement in all the clinical parameters and alkaline phosphatase levels in the gingival crevicular fluid. ($p<0.001$)
2. Intra-group comparison of the Test group from baseline to 4 weeks showed statistically highly significant improvement in all the clinical parameters and alkaline phosphatase levels in the gingival crevicular fluid. ($p<0.001$)
3. Inter-group comparison between Test and Control group presented that Test group had statistically highly significant improvement in all the clinical and biochemical parameters (alkaline phosphatase levels) compared to Control group. ($p<0.001$)

Therefore, it can be concluded that the administration of Polyherbal gel containing Ashwagandha and Ginger as a local drug delivery agent as an adjunct to scaling and root planing has a synergistic effect to scaling and root planning in the treatment of chronic periodontitis. This was ascribed to immunomodulatory, anti-inflammatory, anti-microbial and anti-oxidant properties of Ashwagandha as well as analgesic, anti-inflammatory, antioxidant and anti-bacterial actions of Ginger combined in the form of a polyherbal gel.

Further research using Ashwagandha and Ginger along with long-term follow up periods and large sample sizes should be carried out to verify potential benefits of both the drugs in the field of Local Drug Delivery as an adjunct to scaling and root planning.

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ANNEXURE I – ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1313

CERTIFICATE

This is to Certify that the synopsis titled

EVALUATION AND COMPARISON OF EFFICACY OF POLYHERBAL GEL CONTAINING ASHWAGANDHA AND GINGER AS AN ADJUNCT TO SCALING & ROOT PLANING IN CHRONIC PERIODONTITIS - IN VIVO STUDY.

Submitted by

Dr. _____ **REG. NO. IK0219004** _____ *P. G. Student /*

Staff, Guided by [redacted] *from Department of*

PERIODONTICS *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 15/09/2020

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE II – CONSENT FORM

DEPARTMENT OF PERIODONTICS
KAHER'S KLE V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI

CONSENT FORM

"EVALUATION AND COMPARISON OF EFFICACY OF POLYHERBAL GEL CONTAINING ASHWAGANDHA AND GINGER AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS – AN IN-VIVO STUDY"

PRINCIPAL INVESTIGATOR: DR.

- I, _____, aged _____ years have been informed about my involvement in the study.
- I agree to give my personal details like Name, Age, Gender, Residential Address, Previous and Present dental history and any other details if required for the study to the best of my knowledge.
- I will co-operate with the dentist.
- I will follow the instructions given by the dentist during study.
- I will visit the dentist as and when required for the study, at the given time and date.
- I permit the dentist to utilize the information given and results obtained from this study for presentation and publication without disclosing my identity.
- I have understood the nature of the study and permit the dentist for doing scaling and root planning, collecting gingival crevicular fluid samples and to apply polyherbal gel in periodontal pockets. I have been informed about all the side-effects and limitations of the study.
- I will not claim any returns for my co-operation in this study, even if it is being sponsored by any agency. I am participating with my own will and wish.
- If for any reason I am unable to participate in the study, for reasons unknown, I can withdraw from the study.
- In my full consciousness and presence of mind, after understanding all the procedures and related complications if any, in my vernacular language, I am willing and give my consent to participate in this study.

Date: Address & Ph. No:

Signature:

DEPARTMENT OF PERIODONTICS
KAHER'S KLE'S V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI

CONSENT FORM

"EVALUATION AND COMPARISON OF EFFICACY OF POLYHERBAL GEL CONTAINING ASHWAGANDHA AND GINGER AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS – AN IN-VIVO STUDY"

PRINCIPAL INVESTIGATOR: डॉ.

- मी,_____, वय_____ वर्षे, मला ह्या अभ्यासा बद्धल पुर्ण कल्पना देण्यात आली आहे.
- मी माझी वैयक्तिक माहिती जसे की, नाव, वय, लिंग, पत्ता, मागील व सध्याची दंत उपचाराची माहिती व अन्य तपशील देण्यास सहमत आहे.
- मी दंतचिकित्सकांना त्यांच्या अभ्यासासाठी पुर्ण सहकार्य करेन.
- दंतचिकित्सकांचा अभ्यास चालू असताना, मी त्यांनी दिलेल्या सर्व सुचनांचे पालन करीन.
- मी दंतचिकित्सकांनी सांगितलेल्या वेळेस व तारखेस त्यांच्या अभ्यासासाठी हजर राहीन.
- दंतचिकित्सकांच्या अभ्यासा दरम्यान त्यांनी प्राप्त केलेली माझी सर्व माहिती व अभ्यासाचे परिणाम माझी ओळख लपवून कुठल्याही प्रकाशनात सादर करायला माझी परवानगी आहे.
- मला दंतचिकित्सकांच्या अभ्यासाचे स्वरूप समजले आहे आणि मी त्यांना आवश्यक असलेले जिंजीवल क्रेविक्युलर फ्लूइड चे नमुने व माझ्या पेरीओडोंटल पॉकेट मध्ये जेल चा वापर करण्यास परवानगी देत आहे.
- मी दंतचिकित्सकांच्या अभ्यासात माझे सहकार्य दिल्या बद्धल कोणत्याही परताव्याचा दावा करणार नाही, तसेच जरी त्यांचा अभ्यास कोणत्याही एजन्सि मार्फत प्रायोजित केला असेल तरीही मी परताव्याचा दावा करणार नाही. मी माझ्या स्वतः च्या इच्छेने ह्या अभ्यासात सहभागी होत आहे.
- मी कोणत्याही कारणास्तव अभ्यासात भाग घेऊ शकत नसीन, तर मी ह्या अभ्यासातुन केव्हाही बाहेर पडु शकतो/शकते.
- मी पूर्ण शुद्धीत व माझ्या मनाच्या जागृत अवस्थेत, सर्व प्रक्रिया व त्यांचे क्वचित होऊ शकणारे दुष्परिणाम समजुन, माझ्या मातृभाषेत ह्या अभ्यासात सहभागी होण्यास संमती देतो/देते.

तारीख:-

पत्ता व दूरध्वनी क्रमांक:-
स्वाक्षरी:-

DEPARTMENT OF PERIODONTICS
KAHER'S KLE'S V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI
CONSENT FORM
"EVALUATION AND COMPARISON OF EFFICACY OF POLYHERBAL GEL CONTAINING ASHWAGANDHA AND GINGER AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS – AN IN-VIVO STUDY"

PRINCIPAL INVESTIGATOR: DR.

- ನಾನು,_____ ವಯಸ್ಸಸು_____ ತಮ್ಮ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಮಾಹಿತಿಯನ್ನು ನೀಡುತ್ತೇನೆ .
- ನನ್ನ ಹೆಸರು , ವಯಸ್ಸಸು , ಲಿಂಗ, ವಿಳಾಸ, ಹೆಂಡಿನ ಮತ್ತು ಸದಯದ ಹಲ್ಲಿನ ಇತಿಹಾಸವನ್ನು ತಮ್ಮ ಜ್ಞಾನದ ಅತ್ಯುತ್ತಮ ಅಧ್ಯಯನಕ್ಕೆ ಬೇಕಾಗುವ ಎಲ್ಲ ವಿವರಗಳನ್ನು, ಹಾಗೆ ನನ್ನ ವಯಕ್ತಿಕ ವಿವರಗಳನ್ನು ನೀಡಲು ಒಪ್ಪುತ್ತೇನೆ .
- ನಾನು ತಮ್ಮ ಅಧ್ಯಯನದ ಸಲುವಾಗಿ ಸಹಾಯ ಮಾಡುತ್ತೇನೆ.
- ನಾನು ತಮ್ಮ ದಂತ ಅಡಯನದಲ್ಲಿ ನೀಡಿದ ಸೂಚನೆಗಳನ್ನು ಅನುಸರಿಸುತ್ತೇನೆ.
- ನಾನು ತಮ್ಮ ದಂತ ಅಡಯನದ ಸಲುವಾಗಿ ತಾವು ಸೂಚಿದ ದೀನಾ0ಕ ಮತ್ತು ವೇಳೆಗೆ ಅನುಸಾರವಾಗಿ ತಪ್ಪದೆ ಬರುತ್ತೇನೆ.
- ತಮ್ಮ ದಂತ ಅಡಯನದಲ್ಲಿ ನನ್ನ ಹೆಸರನ್ನು ಅನುಮೋದಿಸದೆ ನನ್ನ ಹಲ್ಲಿನ ಮಾಹಿತಿ ಮತ್ತು ವಯಕ್ತಿತ ಮಾಹಿತಿಯನ್ನು ಪರಸ್ತುತಪಡಿಸುವಲ್ಲಿ ನಾನು ವಪ್ಪುತ್ತೇನೆ .
- ನಾನು ತಮ್ಮ ದಂತ ಅಡಯನದ ಬಗ್ಗೆ ತಿಳಿದಿದದು , ನನ್ನ ಹಲ್ಲಿನ ಮೇಲಿನ ಪಳಿಲಾಕ್ಕನು ತೆಗೆದುಕೊಳ್ಳಲು ಅನುಮತಿಯನ್ನು ಕೊಡುತ್ತೇನೆ ಮತ್ತು ವಡಯರೂ ನೀಡಿದ ಜಲನನ್ನು ಪೊಕ್ಕಳಿಟ ನಲ್ಲಲಿ ಹಚ್ಚಲು ಬಿಡುತ್ತೇನೆ .
- ನಾನು ಆಡಯಯನಕ್ಕಾಗಿ ಬೇರೆ ಯಾವುದೆ ಮೂಲದಿಂದ ಹಣಸಹಾಯ ಬಂದರು ಯಾವುದೇ ಆದಾಯದ ನೆರೀಕಷೆಯನ್ನು ಮಾಡದೆ ಸಹಕರಿಸುತ್ತೇನೆ.
- ನಾನು ತಮ್ಮ ದಂತ ಅಡಯಯನದಲ್ಲಿ ಸ್ವಯೇಯಿಚ್ಚೆಯಿಂದ ಬಾಗಿಯಾಗುತ್ತೇನೆ.
- ನನ್ನ ವಯಕ್ತಿಕ ಕಾರಣಗಳಿಂದ ತಮ್ಮ ದಂತ ಆಡಯಯನಕ್ಕೆ ಸಹಕರಿಸಲು ಸಾಡಯವಾಗದಿಹೋದರೆ ನಾನು ಅಡಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯುತ್ತೇನೆ .
- ನನ್ನ ಪೂರಣ ಪರಜ್ಞಾ ಮತ್ತು ಸಮಯ ಪರಜ್ಞಾ , ಅಡಯಯನದ ಬಗ್ಗೆ ನನ್ನ ಮಾತೃ ಬಾಷೆಯಲ್ಲಿ ತಿಳಿದು , ಅಡಯಯನದಲ್ಲಿ ಒಳಗಾಗುವ ಎಲ್ಲ ವಿಧಾನಗಳು ಮತ್ತು ಸಂಕೀರಣಗಳನ್ನು ತಿಳಿದು , ಅಡಯಯನದಲ್ಲಿ ನನ್ನನನ್ನು ನಾನು ತೊಡಗಿಕೊಳ್ಳಲ್ಲು ಒಪ್ಪುತ್ತೇನೆ .

ದೀನಾ0ಕ : ವಿಳಾಸ ಮತ್ತು ಫೋ.ನ0 :

ಸಹಿ :

ANNEXURE III – PROFORMA

DEPARTMENT OF PERIODONTICS,
KAHER'S KLE's V.K. INSTITUTE OF DENTAL SCIENCES,
BELAGAVI.

"EVALUATION AND COMPARISON OF EFFICACY OF POLYHERBAL GEL CONTAINING ASHWAGANDHA AND GINGER AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS – AN IN-VIVO STUDY"

- Case No: OPD No:
- Name:
- Age: Sex: Occupation:
- Address:
- Chief Complaint:
- Medical History:
- Dental history:

DENTAL PARAMETERS

Number of teeth present: -

Sulcus Bleeding Index (Muhlemann H.R and Son S, 1971)

BASELINE: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

4 WEEK FOLLOWUP: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Plaque Index (PI) (Silness J and Loe H, 1967)

BASELINE: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

4 WEEK FOLLOWUP: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Pocket Probing Depth (PPD)

BASELINE: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

4 WEEK FOLLOWUP: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Clinical Attachment Level (CAL)

BASELINE: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

4 WEEK FOLLOWUP: -

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

PARAMETERS	**BASELINE**		**4 WEEK FOLLOWUP**	
Teeth involved	**Test**	**Control**	**Test**	**Control**
GCF - Alkaline phosphatase levels (IU/L)				
Pocket probing depth				
Clinical attachment level				

SIGNATURE OF THE STUDENT **SIGNATURE OF THE PATIENT**

Patient Information Sheet

- I, Dr. _____ will be conducting this study.
- Patients having chronic periodontitis, following the inclusion and exclusion criteria and who are willing to participate in this study will be selected.
- After informed consent is taken from the patient they will be explained about the procedures and necessary precautions to be undertaken by them before starting, during and after this study.
- In the first appointment, pre-treatment intra oral findings and case history will be recorded along with GCF sample collection after primary removal of plaque and calculus. Treatment protocol will include ultrasonic scaling, root planing using curettes and local application of polyherbal gel in the periodontal pockets of TEST group patients. Periodontal pack will be placed at the site after gel application.
- Oral hygiene instructions will be given to the patient.
- Patient will be recalled after 7 days for removal of the periodontal pack and to assess maintenance of oral hygiene by the patient. (2^{nd} appointment)
- After completion of 4^{th} week, patient will be recalled for follow-up and post treatment findings will be recorded along with collection of GCF.

ANNEXURE IV – MASTER CHART

SR. NO.	Group	PI0	PI4	SBI0	SBI 4	PPD (mm) 0	PPD (mm) 4	CAL (mm) 0	CAL (mm) 4	GCF ALP (IU/µL) 0	GCF ALP (IU/µL) 4
1	T	2	2.5	2	0	5	2	3	0	1614	480.9
	C	1	1	2	0	5	3	3	1.5	1147	726.9
2	T	2	1	1	0	5	3	3	1	843	193.5
	C	1.3	1	0	0	5	3	3	1	802	498
3	T	2.5	1	2.6	1	5.6	2.3	3	1	82.9	27.6
	C	2.6	1	2.6	1	4	3	3	1	276	116.1
4	T	2.6	1	3	1	5	2.5	3	1	290	234.9
	C	2	1	2	1	4	3	2	2	129.9	107.8
5	T	1	1	1	0	5	2.5	3	0.5	160.3	104.2
	C	2.3	1.3	2	1	6	3	4	1	114.2	96.2
6	T	2.6	1	1.75	0	5	3	4	1	779.4	111
	C	2.3	1	2	1	5	3	3	1	754.6	127
7	T	2	1	2	1.6	5	3	3	1	218.4	185.2
	C	1.75	1	2	1	5	3	3	1	608.1	851.3
8	T	2	0.25	1.6	0	5	3	3	1	259.8	158
	C	2	0.25	1.6	1	4	3	2	1	659.1	398
9	T	2	1	2.6	1	5	4.5	3	1	403.5	387
	C	2	1	2	1	4.5	2.5	2.5	1	198	226.6
10	T	2	1	2	1	6	3.5	4	1.5	505.8	145.6
	C	2	1	2	1	5	5	3	3	348.3	119.8
11	T	2	0	2	0	5	3	3	1	552.8	129.9
	C	2	0	1	0	5	3	3	1	530.7	138
12	T	2	1	2	0	4.5	3	2.5	1	1849	478.2
	C	2	2	3	2	5.5	5	3.5	3	1092	138.2
13	T	1	1	2	1	5	2.5	4	1	375.9	116
	C	0	1	2	1	5	4	4	2	259.8	304
14	T	2	0	2	1.3	6	3	4	2	276	181
	C	2	1	2	1	5	4.5	3	3.5	309.6	105.9
15	T	2	1.5	3	0	5	2	4	1	591.5	127
	C	0	2	3	2	5	2.5	4	1.5	420.1	278.2
16	T	2	1	3	2	5	3	3	1	1155	88.4
	C	2	1	3	1	5	3	3	1	138	44.2
17	T	2	0	1	1	6	3	4	2	498	165.6
	C	1.2	1	1	1	5	3	3	1	257	198
18	T	2	1	2	1	5.5	3.5	3.5	1.5	393	193
	C	2	1	2	1	6.5	5	4.5	3	774	364.2
19	T	0	0	2	1	5	2.5	3	1	798	256.2
	C	0	0	2	1	5	3	3	1	846	369.5
20	T	2	1	3	1	5.5	4	4	2	759	365.6
	C	2	1	3	1	5	3	3	1	1012	498.4
21	T	0	1	2	2	5	3	4	2	248.8	90.6
	C	1	2	2	0	5	3	4	2	131.7	85.9
22	T	2	0	0	0	5	4	3	2	265.5	95.2
	C	2	2	0	0	5	3	3.5	3.5	796.6	112.6
23	T	2	1.6	3	2	5	3	3	1	493.1	260.8
	C	2	1	3	2	5	3.6	3.6	1.3	141.5	249.6

ANNEXURE V – AUTHENTICATION CERTIFICATES FOR ASHWAGANDHA LEAVES AND GINGER RHIZOME

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA

A constituent unit KLE Academy of Higher Education & Research
Deemed–to–be–University
Central Research Facility
DRUG AUTHENTICATION REPORT

Submitted By: Dr.

Submitted Date: 21/11/2019 **Date of Issue: 29/11/2019**

SN	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Ayurvedic Name	Scientific Name	Family	Part Authenticated
1	Shunthi	*Zingiber officinale* Roxb.	Zingiberaceae	Rhizome	CRF/Auth / 2019/217	Shunthi	*Zingiber officinale* Roxb.	Zingiberaceae	Rhizome
2	Ashwagandha	*Withania somnifera* (L.) Dunal	Solanaceae	Leaf	CRF/Auth / 2019/218	Ashwagandha	*Withania somnifera* (L.) Dunal	Solanaceae	Leaf

Signature:
Authentication Expert Name: Mr.Ajit Lingayat
Date: 29/11/2019

Signature of Coordinator
ASU Drug Testing Laboratory

ANNEXURE VI – PHYTOCHEMICAL PROFILE OF ASHWAGANDHA

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Reference No:CRF/RM/263/19-20 — Registration Dt:-21/11/2019
Submitted by : — Requisition no:----
Sample : Ashwangandha leaf — Batch No. : NA — Part/Form: Leaf
Product : PLANT — Sample Qty: 50 gm — Report Date:05/12/2019

TEST REPORT

Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

PART	: Leaf
COLOUR	: Greenish
TASTE	: Bitter
ODOUR	: Characteristic

Physico Chemical Standards :

TESTS	RESULTS
Foreign Matter	:Nil
Ash Value	:2.687 %
Acid insoluble Ash	:0.547 %
Water soluble extractive	:23.08 %
Alcohol soluble extractive	:10.24 %

ANALYST

CRF SHAHAPUR, BELGAUM

AUTHORISED SIGNATORY

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA

A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)

(Re Accredited 'A' Grade by NAAC (2nd Cycle) & Placed under Category 'A' by MHRD Govt)

CENTRAL RESEARCH FACILITY

(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Reference No:CRF/RM/263/19-20
Submitted by :
Sample : Ashwanadha leaf
Product : PLANT

Batch No. : NA
Sample Qty: 50gm

Registration Dt:-21/11/2019
Requisition no:----
Part/Form: Leaf
Report Date:05/12/2019

TEST REPORT

Form-50 [See Rule 160-D (f)]

(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening:

TESTS	ALCOHOL	WATER
Test for Carbohydrates	Positive	Negative
Test for Reducing sugar	Negative	Negative
Test for Monosaccharides	Negative	Negative
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Negative
Test for Proteins	Positive	Positive
Test for Amino Acids	Positive	Positive
Test for Steroids	Positive	Positive
Test for Flavonoids	Positive	Positive
Test for Alkaloids	Negative	Negative
Test for Tannins	Positive	Positive
Test for Glycosides:		
A.Cardiac Glycosides	Positive	Negative
B.Anthraquinone glycosides	Negative	Negative
C.Saponin glycosides	Negative	Positive

ANALYST

CRF SHAHAPUR, BELGAUM

AUTHORISED SIGNATORY

ANNEXURE VII – PHYTOCHEMICAL PROFILE OF GINGER (SHUNTHI)

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Reference No:CRF/RM/262/19-20 — Registration Dt:-21/11/2019
Submitted by : — Requisition no:----
Sample : Shunthi — Batch No. : NA — Part/Form: Rhizome
Product : PLANT — Sample Qty: 50 gm — Report Date:05/12/2019

TEST REPORT

Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

TESTS	LIMITS	RESULTS
PART	Rhizome	: Rhizome
COLOUR	Buff coloured	: Buff coloured
TASTE	Agreeable and Pungent	: Agreeable and Pungent
ODOUR	Agreeable and Aromatic	: Agreeable and Aromatic

Physico Chemical Standards :

TESTS	LIMITS	RESULTS
Foreign Matter	Not more than 1%	:Nil
Ash Value	Not more than 6%	:2.687 %
Acid insoluble Ash	Not more than 1.5%	:0.547 %
Water soluble extractive	Not less than 10%	:68.16 %
Alcohol soluble extractive	Not less than 3%	:4.636 %

(Standards reffered above are as per **API**)
* In my opinion the Sample is standard quality

ANALYST

CRF SHAHAPUR, BELGAUM — Shri B. M. Kankanwadi Ayurved Mahavidyalaya • K.L.E. University

AUTHORISED SIGNATORY

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA

A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)

(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)

CENTRAL RESEARCH FACILITY

(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Reference No:CRF/RM/262/19-20 — Registration Dt:-21/11/2019

Submitted by — Requisition no:----

Sample : Shunthi — Batch No. : NA — Part/Form:Rhizome

Product : PLANT — Sample Qty:50gm — Report Date:05/12/2019

TEST REPORT

Form-50 [See Rule 160-D (f)]

(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening:

TESTS	ALCOHOL	WATER
Test for Carbohydrates	Positive	Negative
Test for Reducing sugar	Negative	Positive
Test for Monosaccharides	Negative	Negative
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Negative
Test for Protiens	Negative	Negative
Test for Amino Acids	Negative	Negative
Test for Steroids	Positive	Positive
Test for Flavonoids	Negative	Negative
Test for Alkaloids	Negative	Negative
Test for Tannins	Negative	Negative
Test for Glycosides:		
A.Cardiac Glycosides	Positive	Negative
B.Anthraquinone glycosides	Negative	Negative
C.Saponin glycosides	Negative	Positive

ANALYST

Shri B. M. Kankanwadi Ayurvedic Mahavidyalaya • KLE University

CRF SHAHAPUR, BELGAUM

AUTHORISED SIGNATORY

ANNEXURE VIII – MICROBIOLOGICAL REPORT FOR MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

RESULTS:

Determination of MIC and MBC of emulsion

Table. 1. Minimum inhibitory concentration and Minimum bactericidal concentration of *Withania somnifera* extract

Sr.No.	Samples	MIC and MBC	
		Staphylococcus aureus	*Average*
1	**Stalk solution** (*Withania somnifera* 80mg)	***5 mg***	***6.60 mg***
2		***5 mg***	
3		***10mg***	

Table. 2. Minimum inhibitory concentration and Minimum bactericidal concentration of *Gingiber officinale* extract

Sr.No.	Samples	MIC and MBC	
		Staphylococcus aureus	*Average*
1	**Stalk solution** (*Gingiber officinale 200mg*)	***100 mg***	***130 mg***
2		***100 mg***	
3		***200 mg***	

Table. 3. Minimum inhibitory concentration and Minimum bactericidal concentration of *Withania somnifera and Gingiber officinale* extract

Sr.No.	Samples	MIC and MBC	
		Staphylococcus aureus	*Average*
1	**Stalk solution** (*Withania somnifera and Gingiber officinale extract* 280 mg)	***35mg***	***35 mg***
2		***35 mg***	
3		***35 mg***	

Dr. Bhaskar kurangi