EFFECTIVENESS OF GINGER LEAVES EXTRACT AGAINST STAPHYLOCOCCUS AUREUS AND PROPIONIBACTERIUM ACNE:A NOVEL HERBAL ANTI-ACNE AGENT

Janhavi Borkar[a1*], Amita R. Somalwar[a2], Seema Somalwar[a3]

[a]Nikalas Mahila Mahavidyalaya, Department of Cosmetic Technology, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, 440025, India.

[1]Student, [2]Assistant Professor, [3]Principal

janhaviborkar03@gmail.com, amitasomalwar@gmail.com, seemasomalwar@gmail.com

ABSTRACT:

Acne vulgaris, a common skin affliction, is often associated with bacterial colonization, particularly of Staphylococcus aureus (S. aureus) and Propionibacterium acnes (P. acne), contributing to comedones and inflammatory acnes. In addressing this issue, herbal solutions have gained attention for their potential antimicrobial properties. Ginger leaves are known to possess antibacterial properties; however, their efficacy against these bacteria has not been extensively reported. The current study was undertaken to explore the in-vitro anti-acne potential of ginger leaves against acne-specific bacteria. In the present study, the ginger leaf powder was extracted in a hydroalcoholic solution using the maceration method. A preliminary qualitative phytochemical screening of the ginger extract was conducted. Moreover, the in-vitro anti-bacterial activity of ginger extract against S. aureus and P. acne was assessed using the agar cup plate method. The phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, saponins, sterols, triterpenoids, tannins, and phenoliccompounds. Ginger extract exhibited dose-dependent antibacterial effects against S. aureus at 2.5%, 5%, and 10% concentrations, with inhibition zones of 8±0.25 mm, 9.25±0.15 mm, and 10.39±0.35 mm, respectively. Moreover, the antibacterial properties against P. acne were observed at concentrations of 2.5%, 5%, and 10%, with inhibition zones of 7.5±0.16 mm, 8±0.25 mm, and 9±0.1 mm, respectively. The minimum inhibitory concentration (MIC) of ginger extract, which inhibited the growth of both P. acne and S. aureus, was found to be 5%. These findings suggest that ginger leaves extract could be a potential innovative herbal antibacterial alternative for acne treatment.

Keywords: Gingerleaves extract, Anti-acne, Anti-bacterial, Zone of inhibition, Phytochemical screening.

INTRODUCTON

Acne vulgaris is a chronic inflammatory condition of pilosebaceous units, affecting more than 85% of adolescents and two-thirds of adults aged 18 years and older [1, 2]. It is characterized by seborrhea, open and closed comedones, papules, pustules, and in more severe cases nodules, pseudocysts, and scarring [3]. The Propionibacterium acne, Staphylococcus aureus, and Staphylococcus epidermidis are the major causative bacterial species for acne development [4, 5]. Acne triggers psychological stress due to the occurrence of pustule lesions and post-inflammatory hyperpigmentory scars especially on the face which leads to an emotional impact on an individual's life[6]. Currently, various synthetictopical or systemicagents (keratolytics,anti-inflamatory,antibiotics) are used. However, widespread use of these synthetic drugs and antibiotics has numerous side effects (irritation and immune hypersensitivity) and some have even developed bacterial resistance[2,6,7,8]. In

effectively addressing these challenges, it's essential to highlight the pressing need for advancing research in herbal anti-acne treatment, contributing towards the safety, eco-friendliness and reduced adverse effects compared with synthetic alternatives.

Ginger (ZingiberofficinaleRosc.), a member of Zingiberaceae family, has been very popular to be used not only as spices but also as traditional plant medicine used to treat cough, diarrhea and bacterial infections Ginger (ZingiberofficinaleRosc.), a member of Zingiberaceae family, has been very popular to be used not only as spices but also as traditional plant medicine used to treat cough, diarrhea and bacterial infections Ginger (ZingiberofficinaleRosc.), a member of Zingiberaceae family, has been very popular to be used not only as spices but also as traditional plant medicine used to treat cough, diarrhea and bacterial infections Ginger (ZingiberofficinaleRosc.), a member of Zingiberaceae family, has been very popular to be used not only as spices but also as traditional plant medicine used to treat cough, diarrhea and bacterial infections Ginger (ZingiberofficinaleRosc.), a member of Zingiberaceae family, has been very popular to be used not only as spices but also as traditional plant medicine used to treat cough, diarrhea and bacterial infections Ginger (ZingiberofficinaleRosc.), a member of the Zingiberaceae family, has been very popularly used not only as spices but also as a traditional medicinal plant [9]. Ginger is one of the most utilized herbs and is known to exhibit enormous pharmacological properties including, antifungal, anti-inflammatory, anti-carcinogenic, antithrombotic, antioxidant, hepatoprotective[10, 11,12]. Ginger rhizomes, flowers as well as leaves are enriched with bioactive components (phytoconstituents) like, tannins, phenolic compounds, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids. The phenolic components and terpenes are the key active ingredients which include, gingerols (6-gingerol, 8-gingerol, and 10-gingerol), paradols, shogaols, quercetin, gingerenone-A, zingerone, and 6-dehydrogingerdion. Additionally, ginger contains several terpene components, including α-curcumene, β-bisabolene, β-sesquiphellandrene, α-farnesene and zingiberene [4,9,12,13,14]. Ginger contains potent antibacterial secondary metabolites such as gingerol, paradol, zingiberol, zingiberene, and bisabolene [9,15]. Importantly, gingerol has been reported as an active inhibitor of bacterial species against M. avium and M. tuberculosis. An array of evidence revealed the antibacterial effects of ginger against several bacterial species including, Escherichia coli, Bacillus subtilis and Staphylococcus spp, Trichomonasvasmalis, Salmonella typhi, Streptococcus mutans, Enterococcus faecalis, and Lactobacillus spp. [12,15; 16,17, 12]. Tanweer et al. (2020) [18] recently established the maximum prevalence of 6-gingerol in ginger leaves as compared to ginger flowers and ginger rhizomes. Ginger rhizome extract demonstrated in-vitro antibacterial effects against acne-causing bacteria, P. acne, and S. aureus [9]. However, the putative role of ginger leaves as an anti-acne agent against P. acne and S. aureus has remained unexplored. Consequently, in the present investigation, ginger leaves were selected to explore its in-vitro anti-acne potential against P. acne and S. aureus. common cold, digestive disorders, hypercholesterolemia, heart diseases, lung diseases

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MATERIAL AND METHODS

The ginger leaves were procured from local market of Nagpur, district of Maharashtra, India and authenticated at Department of Botany, R.T.M. Nagpur University.

Extraction of Ginger Leaves by Maceration

The samples of ginger leaves were sorted, washed, and shadedried at room temperature for 15 days. The plant materials were finely

crushed into a powder using an electronic grinder and stored in airtight polyethylene bags at room temperature for further use.Approximately 40 grams of ginger leaves powder were soaked and macerated in 400 ml of 96% hydroalcoholic solvent (ethanol-water) for 3 days with occasional stirring. After extraction, the extract was decanted and filtered through Whatman filter paper. The hydroalcoholic crude extract was obtained by evaporating the solvent using a rotary evaporator and a water bath at 60°C. The hydroalcoholic extract was weighed (3 gm) and then stored in the refrigerator at 4°C until use[19].

Phytochemical Screening (Qualitative Analysis)
Ginger leaves extract was subjected to phytochemical screening for presence of phytoconstituents such as carbohydrates, alkaloids, flavonoids, saponins, sterols, triterpenoids, tannins, and phenolic compoundswith the following standard procedures [4,9,20,21].
Test microorganism :In the present study, the standard bacterial culture of Propionibacterium acne (MTCC-1951) and Staphylococcus aureus (MTCC-96)were obtained in lyophilized form, from Microbial Type Culture Collection (MTCC), Chandigarh, India
Preparation of the media :The Propionibacterium acne and Staphylococcus aureus bacteria were activated by inoculation in the Nutrient culture medium followed by 24 hours of incubation at 37∘C. For preparation of microbial suspension, a 24-hour culture was used [22].

In-vitro anti-bacterial Assays
Determination of minimum inhibitory concentration (MIC)
Minimum inhibitory concentration (MIC) is the lowest concentration of extract that inhibits the growth of test organisms by preventing the appearance of turbidity.

Broth dilution method
The antibacterial activity of hydroethanolic extract of Ginger leaves were evaluated using broth dilution method according to the procedure described by Shaikh et al. [23] with some modifications[5].

Agar cup plate method
The antibacterial activity against S. aureus and P. acne was investigated by agar cup plate method according to the procedure described by Ruth and Miller, 2015[24]with some modifications. Nutrient agar culture medium was poured into the petri plate. After the medium got solidified, 500 μL of each microbial suspension was swabbed on respective agar plates. Then, wells measuring8 mm diameter were punched aseptically with a sterilecork borer. The 100 µL (0.1 ml) of hydroethanolic ginger leaves extracts at2.5%, 5% and 10% concentrations were added into each wells respectively. Tetracycline(0.3 mg/ml) solution was used as a reference standard control. The plates were incubated at 37 °C for 24 hrs. The diameter of the growth inhibition zone was measured in millimeters. This process was repeated in triplicate and the mean diameter of the growth inhibition zone was calculated for different concentrations of the extract.

RESULTS AND DISCUSSION

Preliminary phytochemical screening
Phytoconstituents are the secondary metabolites synthesized naturally by the plants that contributes to its pharmacological properties including antimicrobial against different microbial pathogens (anti-bacterial, antifungal). Hence, phytotherapy has emerged as widely acceptable safe sustainable approach for treatment of several bacterial infections [9, 16]. Therefore, in the present investigation, the bioactive constituents in the hydroethanolic extract of ginger leaves was evaluated using preliminary phytochemical screening (qualitative analysis). The phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, saponins, sterols, triterpenoids, tannins, and phenolic compounds, as depicted in Table 1. The results are in accordance with the previous findings which suggest that presence of different bioactive components in the herbs (flavonoids, alkaloids, phenols and tannins) attributes to its antimicrobial properties [19, 25].
In-vitro Antibacterial/Antiacne potential of ginger leaves extract

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In the present study, the hydroethanolic extract of ginger leaves unveiled significant antibacterial activity in both broth dilution and agar cup plate method. In the broth dilution assay, ginger leaves extract dose-dependently inhibited the growth of P.acne and S.aureus with the increasing concentrations (1% to 8%). The minimum inhibitory concentration (MIC) MIC of hydroethanolic extract of ginger leaves that inhibited the growth of P.acne and S.aureus was observed at 5% concentration relative to control group as evident by absence of turbidity. Table 2 and Table 3demonstrates the MIC of hydroethanolic extract of ginger leaves at different concentrations against S. aureus and P. acne respectively. Moreover, in agar cup plate method, ginger extract displayed dose-dependent antibacterial effects against S. aureus at concentrations of 2.5%, 5%, and 10%, resulting in inhibition zones of 8±0.25 mm, 9.25±0.15 mm, and 10.39±0.35 mm, respectively as shown in Table 4 and Fig.1. Similarly, antibacterial effects against P. acne were observed at concentrations of 2.5%, 5%, and 10%, with inhibition zones of 7.5±0.16 mm, 8±0.25 mm, and 9±0.1 mm, respectively as shown in Table 5 and Fig.2. The results of present study corroborate with previous findings which suggest that ethanolic extracts of ginger rhizome at concentration of 12.5% exhibited antibacterial activity against acne-origin bacteria Staphylococcus and Propionibacteriumspp[9].On the similar lines, study from [25]demonstrated that red ginger was effective against Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus agalactiae. The antibacterial effects of ginger leaves extract have been attributed to phenolic components and terpenes with the major constituents, gingerol, paradol, zingiberol, zingiberene, and bisabolene [9,15]. Substantial evidences revealed the antibacterial effects of ginger against several bacterial species including, Escherichia coli, Bacillus subtilis and Staphylococcus spp, Trichomonasvasmalis, Salmonella typhi[12,15,16,17] Importantly, gingerol has been reported as active inhibitor of bacterial species against M. avium and M. tuberculosis. Recently, Tanweer,[2020][18] proposed that ginger leaves showed maximum prevalence of 6-gingerol as compared to ginger flowers and ginger rhizome. Taken together, in the present study, we speculate that ginger leaves serve as a potential herbal antiacne agent that might attributed to, 6-gingerol.

CONCLUSION

The present study for the first time unveils the novel potential of ethanolic extract of ginger leaves as an antiacne agent against acne causing bacteria, P.acne and S.aureus. We suggest that ginger leaf extract holds promise as an innovative herbal alternative for acne treatment due to its antibacterial properties.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Sr.no	Phytochemicals	Name of the test	Results
1.	Carbohydrates	Molisch's test	+
		Seliwinoff's test	+
		Fehling's test	+
2.	Steroid and triterpenoids	Salkowski test	+
		Sulphur powder test	+
3.	Alkaloids	Dragendorff's test	+
		Mayer's test	+
		Wagner's test	+
		Hager's test	+
		Tannic acid test	+
4.	Tannins and Phenolic Compounds	Ferric Chloride test	+
		Gelatin test	+
		Alkaline Reagent test	+
5.	Flavonoids	Shinoda Test	+
		Alkaline Reagent Test	+
		Zinc Hydrochloride Test	+

Table 1: Preliminary phytochemical testing of ginger leaves

Table 2.Evaluation of minimum inhibitory concentration (MIC) against and Staphylococcus aureus using broth dilution method.

Sr. no	Concentration of ginger leaves extract	Amount of medium (ml) (Broth+P.acne)	Total volume of solution (ml)	Turbidity
1.	1%	9 ml	10 ml	+++
2.	2%	8 ml	10 ml	++
3.	3%	7 ml	10 ml	++
4.	4%	6 ml	10ml	+
5.	5%	5 ml	10 ml	-
6.	6%	4 ml	10 ml	-
7.	7%	3 ml	10 ml	-
8.	8%	2 ml	10 ml	-
9.	Control (without active)	10 ml (Broth only)	10 ml	-

Table.3Evaluation of minimum inhibitory concentration (MIC) against and Propionibacterium acnebacteria using broth dilution method.

Sr. no	Concentration of ginger leaves extract	Amount of medium (ml) (Broth+S.aureus)	Total volume of solution (ml)	Turbidity
1.	1%	9 ml	10 ml	+++
2.	2%	8 ml	10 ml	++
3.	3%	7 ml	10 ml	++
4.	4%	6 ml	10 ml	+
5.	5%	5 ml	10 ml	-
6.	6%	4 ml	10 ml	-
7.	7%	3 ml	10 ml	-
8.	8%	2 ml	10 ml	-
9.	Control (without active)	10 ml (Broth only)	10 ml	-

Table 4: Dose-dependent antibacterial activity of Ginger leaves (Test) against Staphylococcus aureus using agar cup plate method

Hydro ethanolicginger leaves extract (Test)	**Concentration**			**Control (Tetracycline)**
Zone of inhibition (Mean ± SD)	**2.5%**	**5%**	**10%**	**0.3 mg/ml**
	8±0.25 mm	9.25±0.15 mm	10.39±0.35 mm	11.5 ±0.34mm

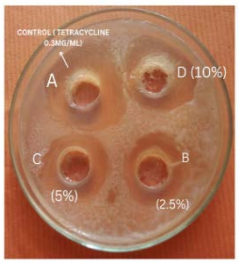

Figure 1. The zone of inhibitions of ethanolic extract of Ginger leaves against Staphylococcus aureus at different concentrations using agar cup plate method
A-Standard control Tetracycline (0.3mg/ml)
B-Ginger leaves extract (2.5%)
C-Ginger leaves extract (5%)
D-Ginger leaves extract (10%)

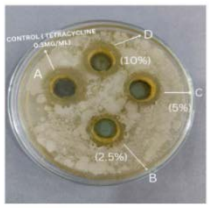

Figure 2. The zone of inhibitions of ethanolic extract of Ginger leaves against Propionibacterium acneat different

A-Standard control Tetracycline (0.3 mg/ml)
B-Ginger leaves extract (2.5%)
C-Ginger leaves extract (5%)

Hydroethanolic ginger leaves extract (Test)		**Concentration**		**Control (Tetracycline)**
	2.5%	**5%**	**10%**	**0.3 mg/ml**
Zone of inhibition (Mean ±SD)	7.5±0.16 mm	8±0.25 mm	9±0.1 mm	11±0.55mm

Table 5: Dose-dependent antibacterial activity of Ginger leaves (Test) against Propionibacterium acneusing agar cup plate method

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