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Phytochemical Screening, Antimicrobial and Antifungal Activities of Zingiber officinale Collected from Dang and Arghakhanchi Districts of Nepal

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Abstract

Phytochemical screening, Antimicrobial and antifungal activities of rhizomes extracts of Zingiber officinale, grown in Dang and Arghakhanchi districts of Nepal, in different solvents is still not understood clearly. The rhizomes of the plant were collected, dried, chopped, powdered and subjected for extraction using maceration technique. The solvent methanol (MeOH), dichloromethane (DCM) and ethyl acetate (EtOAc) were used for the extraction of secondary metabolites. Phytochemical screening, antimicrobial activity and antifungal activities were gauged in the extract of different solvents. Efficient antimicrobial activity, especially against S. aureus and P. aeruginosa, was observed in ethyl acetate (EtOAc) extract of sample collected from Dang district (S_2). Strong antifungal activities were exhibited in methanolic extract sample of both Arghakanchi (S_1) and Dang districts (S_2). Extracts of all ginger samples do not show any zone of inhibition against E. Coli. Rhizome extracts of both samples (S_1 and S_2) show potentiality against microbial and fungal infections.

Keywords Antimicrobial, Antifungal, Ethyl acetate, Ginger (Zingiber officinale), Phytochemical screening

Introduction

Zingiber officinale, commonly referred to as ginger, is a tropical perennial plant of Zingiberaceae family. The crop thrives in temperatures between 19°C - 28°C and requires humidity levels of 70% to 90%, (Adhikari & Bhandari, 2022). Entire plant has a pleasant aroma. Grown-up ginger roots are tough and fibrous. Zingiberene plants possess potent aromatic and curative qualities. Natural products have remarkable structural diversity and distinct biological activities. They are classified based on their biological function, biosynthetic pathway, or origin (Abozenadah et al., 2017).

Ginger (*Zingiber officinale*) is rich in essential compounds, including phenolic substances like gingerols, shogaols, and paradols, (Bhattarai et al., 2018). Ginger is

recognized having anti-aging properties. Free radicals generated during oxidation play key roles in triggering various long-term illnesses i.e., cardiovascular diseases, diabetes, inflammatory conditions, Alzheimer's disease, rheumatoid arthritis, and cancer (Aher & Wahi, 2011). Antioxidants are key molecules to repair oxidative damage of cells. Secondary metabolites, i.e., phenolic compounds, alkaloids, flavonoids delay initiation of lipid peroxidation, (Al-Fatimi et al., 2007).

It's raw or processed underground rhizome is used as a spice (Chen et al., 2007). Rhizomes can be used to make tea. The juice of ginger rhizomes is highly effective to flavor seafood, mutton, snacks, and stews. Dried ginger powder is used in various dishes, sweets, cookies, crackers, cakes, and carbonated non-alcoholic beverages. *Z. officinale* is also valued as herbal medicine for treating various health issues, including diabetes, nausea, migraines and sialagogue effects, i.e., promoting saliva production (Masuda et al., 2004).

Synthetic inhibitory drugs have been associated with certain percentage of adverse side effects (Fatemeh Jamshidi-Kia et al., 2018; Bhattacharjee et al., 2017; Yuan et al., 2016). Dugs derived from natural products have promising alternative for disease treatment having insignificant or no side effects.

Comprehensive profiling of the biological activities of ginger from selected regions has yet to be undertaken. The aim of this study is to evaluate antimicrobial and antifungal activities of plant extracts.

There is global shift towards herbal remedies due to safe and cost-effective natural approach to prevent and cure disease. Nepal's diverse climate, soil texture, total organic and inorganic carbon, and soil pH offer plant biodiversity having vast reservoir of secondary metabolites. Natural products derived from Nepal's biodiversity present a promising avenue for discovering novel compounds and developing new therapeutic agents.

Materials and Methods

Rhizomes of the Gingers were collected from Arghakhanchi and Dang districts of Nepal in June 2024. Ginger rhizomes were washed with distilled water, chopped into pieces and air dried. Dried pieces were ground in a mortar, sieved through a 40-mesh sieve and packed into plastic zipper bag and kept in desiccator until further analysis.

Extraction of secondary metabolites in methanol, dichloromethane and ethyl acetate was carried out as mentioned in (Shrestha et al., 2015). Reagent preparation and phytochemical screening were carried out as mentioned in (Mager et al., 2024; Mishra et al., 2012). Antibacterial activity and antifungal activity were put into action as explained in (Sabudak, et al., 2013; Tahoma et al.; Dingle et al., 1953; Meyer, et al., 1982; Mao et al., 2019). S_1 and S_2 refers to the samples of Arghakhanchi and Dang districts. MeOH, EtOAc and DCM refers to methanol, ethyl acetate and dichloromethane respectively as solvent for extraction. All experiments were carried out in M.Sc. chemistry laboratory

at Birendra Multiple Campus, Chitwan, a sister organization of Tribhuvan University, Nepal.

Results and Discussions

The yields of Ginger rhizomes extract were differed on location of cultivation and the solvent used as mentioned in Table 1.

Table 1. Yield of Ginger Rhizomes extracts.

Ginger sample	The dry weight of ginger (g)	Yield (g)	%Yield
S_1(MeOH)	400.00 g	18.20 g	4.55
S_2(MeOH)	400.00 g	17.30 g	4.32

Table 2. Yield of extract in different solvent

Ginger sample	S1(EtOAc)	S1(DCM)	S2(EtOAc)	S2 (DCM)
Yield (g)	4.50 g	3.70 g	4.30 g	3.40 g

Phytochemical screening

Phytochemical screening was carried out on the selected ginger extracts as mentioned in Magar et al., 2024 and the results are mentioned in Table 3.

Table 3. Phytochemical screening of ginger extracts of all ginger samples

Phytochemicals	S_1(EtAc)	S_1 (MeOH)	S_2(EtOAc)	S_2 (MeOH)
Basic alkaloids	-	+	+	++
Glycosides	+	++	+	++
Saponins	+	++	++	+++
Flavones	+	++	-	++
Tannin	++	+	++	+
Diterpenes	++	+	++	-
Steroids & terpenoids	++	_	+++	+
Cartenoids	++	+	++	-
Polyphenol	+	++	+	+
Carbohydrates	_	+	_	+
Quinone	+	-	+	+
Volatile oil	++	-	+++	+
Coumarin	+++	+	++	-

Note: (++) strongly positive, (+) weakly positive, (-) negative (absence of required ppt/ color).

Antimicrobial activity

The antimicrobial activity of ginger extracts was assessed by measuring the diameter of the zone of inhibition (ZOI) formed against specific bacteria and fungi (Joshi, P. and Khanal, S. 2021). Antimicrobial activity was carried out on gram-positive bacterium (Salmonella typhi aureus) and a gram-negative bacterium (*Escherichia coli*, *Salmonella Typhi*, *Klebsiella pneumonia*). Zone of inhibition (ZOI) created by extract (sample) was measured as mentioned in the table 4.

Neomycin (25.00 µg/mL) was used as the positive control. Among the two strains, i.e., gram-positive bacteria were more effectively inhibited than gram-negative bacteria. This difference proves unique attributes of outer membrane found in gram-negative bacteria, which prevents the extract from being entered into the cell, a feature not present in gram-positive bacteria, (Shirin, et al., 2010; Joshi, P. and Khanal, S. 2018; Sivasothy et al., 2011).

Table 4. Antibacterial activity of ginger extract.

S. N.	Ginger sample	Bacteria	ZOI (mm) of extract 25.00 µg/mL	ZOI (mm) of neomycin control (25.00 µg/mL)
1	S_1(EtOAc)	*E coli*	-	18.00
		K. pneumonia	7.00 ± 0.00	20.00
		P. aeruginosa	-	16.00
		S. aureus	15.40 ± 0.20	18.00
		S. typhi	9.00 ± 0.00	22.00
2	S_1(MeOH)	*E coli*	-	18.00
		K. pneumonia	11.00 ± 0.00	20.00
		P. aeruginosa	-	16.00
		S. aureus	15.80 ± 0.50	18.00
		S. typhi	12.30 ± 0.20	22.00
3	S_1(DCM)	*E coli*	-	18.00
		K. pneumonia	-	20.00
		P. aeruginosa	-	16.00
		S. aureus	11.20 ± 0.30	18.00
		S. typhi	10.50 ± 0.80	22.00
4	S_2(EtOAc)	*E coli*	-	18.00
		K. pneumonia	9.60 ± 0.40	20.00
		P. aeruginosa	-	16.00
		S. aureus	15.50 ± 0.10	18.00
		S. typhi	13.70 ± 0.50	22.00

S. N.	Ginger sample	Bacteria	ZOI (mm) of extract 25.00 µg/mL	ZOI (mm) of neomycin control (25.00 µg/mL)
5	S_2(MeOH)	*E. Coli*	-	18.00
		K. pneumonia	7.60 ± 0. 60	20.00
		P. aeruginosa	6.00 ± 0.00	16.00
		S. aureus	16.30 ± 0.70	18.00
		S. typhi		22.00
6	S_2(DCM)	*E Coli*	-	18.00
		K. pneumonia	6.00 ± 0.00	20.00
		P. aeruginosa	4.00 ± 0.00	16.00
		S. aureus	12.00 ± 0.00	18.00
		S. typhi	9.00 ± 0.00	22.00

Well size = 6.00 mm, Zone of inhibition of DMSO (negative control) = 6.00 mm**,** (-) the sign indicates the absence of antibacterial activity.

Antifungal activity

Antifungal activity was carried out against *Fusarium oxysporium, Aspergillus flavus, Candida ablicans, Candida parapsilosis*. The antifungal activity of the plant extracts was assessed by measuring the diameter of the zone of inhibition (ZOI) against specific fungi and the result is mentioned in the table 5. Amphotericin B (25.00 µg/mL) was employed as the positive control. Ginger extracts of S_1(EtOAc), S_1(MeOH), S_2(EtOAc), S_2(MeOH) exhibited a zone of inhibition against *Candida albicans*, *Candida parapsilosis*, and *Aspergillus flavus*. S_1(DCM), S_2(DCM) samples shows a zone of inhibition for *Fusarium oxysporum*. Above data concludes that ginger extract can be used as antifungal activity. Extracts of samples S_1(EtOAc) and S_2(EtOAc) show the highest ZOI in *Candida parapsilosis* (14.00 mm) and (11.00 mm) respectively. Ginger extracts of samples S_1(MeOH) and S_2(MeOH) shows the same ZOI for *Candida ablicans* (13.00 mm). This result is consistent with the findings reported by Rawal et al. (2016) and here different solvent extracts were used and antifungal activity increased with increasing concentration (Sivasothy et al., 2011).

The observed antifungal activity is due to the presence of alkaloids and flavonoids. Al-Nafie et al. (2024) demonstrated that the growth of Aspergillus spp. obtained from rice seeds were inhibited by secondary metabolites such as alkaloids, flavonoids and terpenoids. These are the key components for antifungal properties. (Modaressi et al., 2013; Faisal Z. 2019; Kumari S. 2016; Gacem et al., 2019). This study investigated that ginger rhizome extract is capable of suppressing various pathogenic microorganisms.

Table 5. Antifungal activity of ginger extracts.

S. N.	Ginger sample	Fungi	ZOI (mm) of extract 50.00 µg/ mL	ZOI (mm), Amphotericin B, (25.00 µg/mL)
1	S_1(EtOAc)	*Fusarium oxysporum*	-	20.00
		Aspergillus flavus	8.40 ± 0.30	20.00
		Candida ablicans	1.50 ± 0.21	19.00
		Candida parapsilosis	14.00 ± 0.40	16.00
2	S_1(MEOH)	*Fusarium oxysporum*	-	20.00
		Aspergillus flavus	7.00 ± 0.61	20.00
		Candida ablicans	13.00 ± 0.18	19.00
		Candida parapsilosis	9.00 ± 0.33	16.00
3	S_1(DCM)	*Fusarium oxysporum*	8.00 ± 0.37	20.00
		Aspergillus flavus	6.80 ± 0.41	20.00
		Candida ablicans	-	19.00
		Candida parapsilosis	-	16.00
4	S_2(EtOAc)	*Fusarium oxysporum*	-	20.00
		Aspergillus flavus	7.30 ± 0.61	20.00
		Candida ablicans	8.20 ± 0.28	19.00
		Candida parapsilosis	11.00 ± 0.35	16.00
5	S_2(MEOH)	*Fusarium oxysporum*	-	20.00
		Aspergillus flavus	9.30 ± 0.45	20.00
		Candida ablicans	13.00 ± 0.21	19.00
		Candida parapsilosis	9.00± 0.28	16.00
6	S_2(DCM)	*Fusarium oxysporum*	6.00± 0.30	20.00
		Aspergillus flavus	-	20.00
		Candida ablicans	-	19.00
		Candida parapsilosis	-	16.00

Conclusions

Glycosides, Saponins, tannin, and polyphenol are present in all ginger extracts. Methanol (MeOH) extracts more polar compounds like glycosides, saponins, flavones, polyphenols, and carbohydrates, while ethyl acetate (EtOAc) is more effective for non-polar compounds such as volatile oils, diterpenes, carotenoids, and coumarin, with both solvents showing varying levels of phytochemical presence across the S_1 and S_2 samples.

Ginger sample S_2(EtOAc) collected from Dang district has a good scavenging ability as compared with other samples. Extracts of all ginger samples do not show any zone of inhibition against *E. Coli*. Extract of S_2(MeOH) shows maximum ZOI for S. *aureus,* and *P. aeruginosa.* Ginger *Extracts* of sample S_1(EtOAc), S_1(MEOH), S_1(DCM), S_2(EtOAc) and sample S_2(DCM) show the highest ZOI for *S. aureus* (15.40 mm), (15.80 mm), (11.20 mm) (15.50 mm) and, (12.00 mm) respectively, and that of sample S_2(EtOAc) shows the highest ZOI for *Salmonella typhi (*13.70 mm).

All ginger extracts displayed a ZOI against *Candida parapsilosis*, *Candida albicans*, and *Aspergillus flavus*, but no inhibition was observed against *Fusarium oxysporum* whereas zone of inhibition is shown by extracts of S_1(DCM), S_2(DCM). Extracts of S_1(EtOAc) and S_2(EtOAc)show the highest ZOI in *Candida parapsilosis* (14.00 mm) and (11.00 mm) respectively.

Conflicts of interest: The authors have declared no conflict of interest.

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