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Article

Therapeutic potential and bioactive phenolics of locally grown Pakistani and Chinese varieties of ginger in relation to extraction solvents

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Current study compares the Therapeutic/nutra-pharmaceuticals potential and phenolics profile of Pakistani grown Pakistani and Chinese varieties of ginger. Crude yield of bioactive components from the varieties tested, using different extraction solvents including chloroform, ethyl acetate, ether, methanol, ethanol and distilled water. The crude bioactives varied from 14.1-82.5%. The highest extraction yield was noted for Pakistani species. The HPLC analysis revalued significant amounts of phenolics including vanillin, protocatechuic, vanillic, ferulic, sinapinic and cinnamic acids. The highest anti-inflammatory activity was shown by ethanolic extract of Pakistani variety (IC_{50}: 26.5±1.8) whereas Chinese variety exhibited potent anticancer potential against MCF-7 cell line (Inhibition: 91.38 %). The Chinese variety in general showed higher phenolics and anticancer, while the Pakistani exhibited higher anti-inflammatory activity. Pakistani grown ginger and ethanolic extract of Chinese ginger showed highest antimicrobial activity against *Pseudomonas aeruginosa* 18.0±0.02 & 15.00±0.02 mm respectively. Minimum results obtained with water for both varieties of ginger with range of 7.2±0.22 and 6±0.07 respectively. Moreover, the phenolics composition, anti-inflammatory, antibacterial and anticancer activities of both tested varieties of ginger were notably affected as a function of extraction solvents. Our findings advocate selection of appropriate solvent for recovery of effective phenolic bioactive compounds from ginger verities to support the Nutra-pharmaceutical formulation.

Keywords: Ginger. Phenolic acids. Anticancer agents. Anti-inflammatory. Antibacterial activity.

INTRODUCTION

Ginger (*Zingiber officinale*), is belonging to a member of *Zingiberaceae* family and is popular as a valued spice and folk medicine. It has been used as a traditional medicine to treat different human ailments since prehistoric times. Ginger is a rich source of a wide array of nutrients and high-value natural antioxidant and anti-inflammatory agents such as phenolics, gingerol, zerumbone and shogaol among others and is thus recognized as a functional food (Singh, Patel, Bachle, 2014). The major active ingredient in ginger rhizome is gingerol which is also responsible for the characteristic pungent smell of ginger as well as various pharmacological properties such as anti-tumor, anti-inflammatory, anticancer, antioxidant, anti-platelet, cardio tonic and anti-emitic effects (Moa *et al.,* 2019).

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Ginger and its dynamic ingredients suppress growth and persuade apoptosis of diversity of cancer types counting skin, brain, colon, oral, breast, cervical, renal, prostate, pancreatic, gastric, liver, and ovarian cancer. In addition to anticancer potential, the secondary metabolites of ginger have also been found to exhibit antioxidant, anti-inflammatory and anti-mutagenic properties (Prasad, Tyagi, 2015; Singh *et al.,* 2016).

Anticancer effects of ginger can be credited to the occurrence of many pungent compounds known as vallinoids including gingerol and paradol, shogaols, zingerone, and galanals which are potent apoptosis inducers of human T lymphoma Jurkat cells (Agrahari *et al.,* 2015). Characteristically, ginger and its constituent mainly gingerol which are more effective against ovarian cancers particularly *in-vivo*. Ginger inhibits necrosis factor kappa-B (NF-kB) and also interleukin-8 (IL-8) inhibitions (Rhode *et al.,* 2007). Ginger extracts reduced the elevated expression of tumor necrosis factor - alpha (TNF-α) and NF-κB in liver cancer of rats (Habib *et al.,* 2008). The anti-inflammatory effect of ginger has been known for centuries. The effectiveness of either ginger or of compounds isolated against inflammation and its mediators in human cells has also been documented (Ali *et al.,* 2008). The anti-inflammatory activity of ginger has been studied to occur by suppression of prostaglandin (PG) synthesis and via interference in cytokine signaling (Al-Awwadi, 2017). An important compound namely 6-gingerol acts as an anti-inflammatory agent and is useful for the treatment of inflammation without interfering with antigen presenting function of macrophages. The ginger extracts have also been described to prevent the induction of numerous genes involved in the inflammatory response such as chemokines, genes encoding cytokines, and the inducible enzyme COX-2 (Kumar *et al.,* 2013).

Ginger is used in the pharmaceutical industry and as an additive in foods in China and other parts of the world. Pakistan is a developing country with limited sources of hygienic and treatment parameters. In this regard an effort was made to find out therapeutic potential through natural products as the country is blessed with huge biodiversity of natural flora. Additionally, a comparison with Chinese ginger variety was planned to measure phytochemical variation effect due to climatic and region differences.

Nature has always served as a vital source of medicinal agents. In this direction, a large number of modern drugs have been derived from natural sources (Jyotsna, Neelam, Viveka, 2017). Plants possess special status in folk medicine systems of several civilizations and are valued as a rich pool of natural medicinal agents. The plant based natural bioactive components such as phenolics, alkaloids, terpenoids and biopeptides have multiple therapeutic and biological effects such as antimicrobial, antioxidant, antidiabetic, anticancer and anti-tumor properties (Rahmani, 2014; Williams *et al*, 2004). The current study was deliberate to recover the maximum quantity of bioactives in relation to different solvents from two common varieties of ginger used in Pakistan. The pharmacological potential of extracted bioactives was accessed. This study provides insight knowledge to researchers, scholars and nutra-pharmaceutical industries to get maximum amounts of these bioactives and better variety for their formulation of different recipes/medication as anticancer, anti-inflammatory and antibacterial actives etc. The findings can be used in pharmaceutical products formulation and functional foods/nutra- pharmaceutical to strength the Nutra-pharma industry.

MATERIAL AND METHODS

Ginger material

The samples of two varieties of ginger (*Zingiber officinale*): a Pakistani grown and a Chinese variety that were cultivated in Pakistan were purchased from the local market of Sargodha-Pakistan (Figure 1). All chemicals and solvents used were highly purified and purchased of analytical mark and obtained from Merck or Sigma-Aldrich Chemicals Corporation, Germany.

A B

FIGURE 1 - (A) Pakistani ginger and **(B)** Chinese ginger.

Extraction of bioactive components

The whole ginger root was cut off into small pieces, dried in shade and then ground in an electrical grinder. The homogenous powdered sample (10g) of both the varieties of ginger was distinctly mixed with 100mL of each of extraction solvents counting MeOH, EtOH, $CHCl_3$, EtOAc, petroleum ether and H_2O. The samples were subjected to shaking in an orbital shaker (Optima OS-752) for 8 hours at 200 rpm at room temperature. Ratio of sample and solvent was kept as 1/10 (w/v). After the extraction, the extracts were sieved to remove the residues which were then re-extracted in the alike manner with fresh solvent. The 3 extraction was pooled and extract solvent was condensed off under abridged pressure on rotary evaporator apparatus (Heidolph HB digital, LABORTA, 4001-Efficient). The crude concentrated semisolid extracts produced were kept at 4ºC before employed for further experiments (Sultana, Anwar, Ashraf, 2009). The percentage yield was obtained by common percentage yield formula through division of actual dry weight of plant material divided by extract obtained weight to multiply with 100.

HPLC analysis of phenolics

HPLC analysis was performed by following the procedure reported by (Hussain *et al.,* 2013) with slight modifications. HPLC (SCM1000, Thermo Finnigan, California USA) having ODS (C18) reversed phase column **(**250 mm x4.6mm; 5um**)** was used for separation of phenolics in different extracts. A solvent system consisting [solvent 1 (Acetonitrile/ MeOH, 70:30%) and solvent 2 (0.5% glacial acetic acid with purified water) was used as a mobile phase at a constant flow rate of 1 mL/min in gradient mode. A 20-µL sample was injected via micro syringe into the column. Through the analysis of UV-Vis spectra of the individual phenolic acid standards and vanillin, different wavelengths were chosen for detection of compounds using the RP-HPLC-DAD. The targeted phenolic acids mainly exhibited λ max at 275 nm. The analytes/compounds were identified by matching their retention time and spiking the samples with those of pure standards whereas quantitative estimation was done based on external calibration curve.

Anti-inflammatory activity

Oxidative Burst assay was performed by using chemiluminescence technique as described by (Helfand, Werkmeister, Roder, 1982). Briefly 25 µL of diluted whole blood Hanks balanced salt solution, containing calcium chloride and magnesium chloride (HBSS++, Sigma, St. Louis, USA) was incubated with 25 µL of three different concentration of extracts (1, 10 and 100µg/mL) each in triplicate. Control wells received HBSS++ and cells, but no sample. Test was performed in white half area 96 well plates (Coster, NY, USA), which was incubated at 37°C for 15 minutes in the thermostat chamber of luminometer (Labsystems, Helsinki, Finland). After incubation, 25 µL of serum opsonized zymosan (SOZ) (Fluka, Buchs, Switzerland) and 25 µL of intracellular reactive oxygen species (ROS) detecting probe, luminal (Research Organics, Cleveland, OH, USA) were added into each well. Only HBSS++ was added to blank wells. The level of the ROS was recorded in luminiometer in terms of relative light units (RLU). Ibuprofen was used as standard for the assay (IC_{50}= 11.2±1.9).

Anticancer potential

MCF-7cell lines were cultured in Dulbecco's modified Eagle medium (containing 10% fetal bovine serum) in 75 mL flasks and kept in 5% CO_2 incubator at 37°C. Upon confluence, cells were harvested and plated overnight in 96-well tissue culture treated flat bottom plates (seeding density 8,000 cells/well for MCF-7 in 100 µL medium). Afterwards, the extracts were added in triplicate at 50 µg/mL concentration and incubated for 48 hours. Then 20 µL of MTT solution (0.5mg/mL in PBS) was added to each well and incubated at 37°C for 3 hrs. After re-incubation, the medium was removed and 100 µL of DMSO was added to each well. The cells were agitated on orbital shaker and absorbance was taken at 570 nm using micro-plate reader (spectraMax plus, Molecular Devices, CA, USA). The percent inhibition or decrease in viable cells was calculated by following formula:

$$\text{Hemolysis}(\%) = \frac{\text{Absorbance (sample)} - \text{Absorbance(negative control)}}{\text{Absorbance (positive control)}} \times 100$$

The extracts that showed ≥ 50% inhibition was further evaluated for IC_{50} calculation. The 20mM stock solution of selected extracts was diluted to a working concentration of 50 µM and then further serial dilutions were made in order to get less than 50% inhibition. The IC_{50} was then calculated by using EZ-fit5 software. The standard drug used in MTT assay was doxorubicin (Helfand, Werkmeister, Roder, 1982).

Antimicrobial activity

Antimicrobial activity was done by disc diffusion method against four bacterial strains; *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Ciprofloxacin drug was used as standard. In this method, first of all nutrient agar was prepared. This medium was heated, mixed and then autoclaved. When the medium was cooled, 100 mL inoculum was added in it. Then it was transferred into sterilized petri plates and mixed. Onward, Wicks paper discs of 6mm were laid flat on medium and each disc was loaded with 100 µL extract. Petri plates were placed in the incubator at 37°C for 24 hours to show antibacterial activity. Inhibition zone was formed for the extracts that showed the antibacterial activity. Zones were measured by the zone reader in mm.

Statistical analysis

The data was expressed as mean values ±SD for triplicate measurements. Analysis of variance was performed using 2-way ANOVA. Significant difference of means among extraction solvents and difference between two ginger varieties were considered at $P < 0.05$. The statistical analysis was done by using SPPS version 22.0.0.0 software.

RESULTS AND DISCUSSION

Different solvents were used for the extraction of the phenolic components from two ginger varieties tested. The extraction yields of bioactive components with different solvents are shown in (Figure 2). Maximum crude yield was obtained with distilled water for both varieties, while

minimum yield was displayed for ether extracts. Based on the extraction yield results, the extraction efficacy of different solvents was found to be in following order: distilled water > methanol > ethanol >ethyl acetate > chloroform > petroleum ether. While with regard to variety, % extract yield of Pakistani grown ginger was greater than Chinese ginger. The results showed that the effect of different solvents and samples of two gingers on percentage extraction yield is significant ($P < 0.05$). It was observed that the nature and concentration of the extraction solvent played a crucial role in the yield of extractable phenolic. It has been observed that phenolic antioxidants have polar nature and are more efficiently extracted in polar solvents (Sajid *et al.,* 2012).

Our findings show similarity with (Arawande, Akinnusotu, Alademeyin, 2018) who reported a higher extraction yield of phenolic from ginger and turmeric was obtained with distilled water. Contrary to present research findings, a previous study reported by (Sharif, Bennett, 2016) that a higher extraction yield from ginger was recorded with ethanol. Differences in the result of extraction yield in the present study may be attributed to various factors such as climatic condition of region, ginger variety and efficacy and nature of extraction solvent (Gull *et al.,* 2012). However, the findings need to be validated via *in-vivo* models that could pave the way for usage of extracted natural product/s from Ginger as health and wellness products.

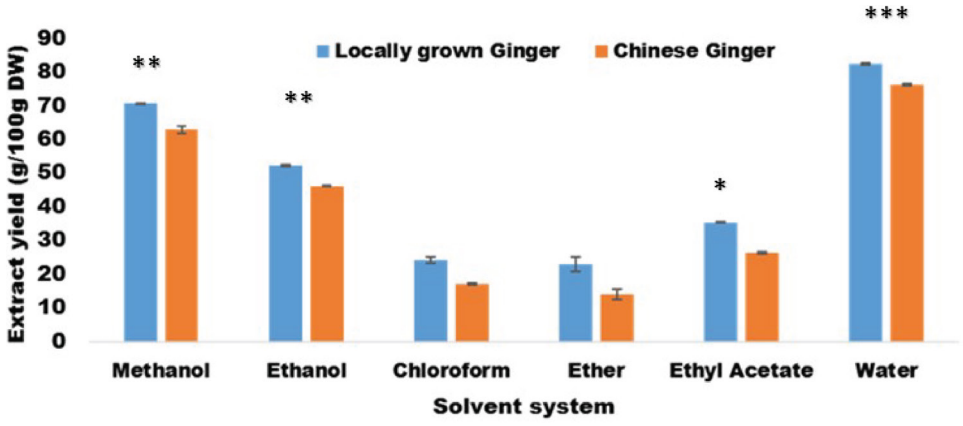

FIGURE 2 - Extraction yields (g/100g) of bioactive extracts from two varieties of ginger (*Zingiber officinale*) with different solvents. All values were taken in triplicate ($n = 3$). There was significant difference observed in all solvent's extraction having $p < 0.05$. The *, **, *** indicates the level of significance (< 0.05 or < 0.01 or < 0.001) among extraction solvents.

HPLC analysis of phenolic

Targeted phenolic in ginger extracts were analyzed quantitatively and qualitatively by HPLC. Standard phenolic acids used for analysis were: vanillin and cinnamic, p-coumaric acid, ferulic, sinapic and protocatechuic acids. The amount of total and individual phenolic acids in Pakistani and Chinese varieties of ginger with MeOH, EtOH, ether, EtOAc, $CHCl_3$, and distilled water are shown in (Table I) respectively. Total phenolic acids in the extracts of two varieties ranged from 94.1 to 7535.9 mg/kg. The highest phenolic content was found in $CHCl_3$ extract of Chinese ginger i.e., 7535.9 mg/kg and lowest in EtOAc extract of Chinese ginger i.e., 94.1mg/kg. While maximum yield of phenolics in Pakistani ginger was obtained with EtOAc i.e., 3156.3 mg/kg and minimum yield was obtained with water (386.46 mg/kg). Overall, methanol proved to be a good choice to get maximum phenolics in Chinese ginger while in Pakistani ginger ethanol solvent offered maximum phenolics. Vanillin was found to be abundant in both the varieties with higher concentration in Chinese ginger. As far as the contents of coumaric acid was concerned, higher values were obtained from Pakistani ginger compared to Chinese ginger, revealing significant ($p < 0.05$) variations among solvents and two ginger varieties in (Table I).

Through literature it is evident that ginger grown in different origins contains diversity in terms of Phyto-constitutes. The ginger grown in China has high contents of Sesquiterpenes which is somehow different from African ginger that has a higher amount of Z-γ-bisabolene but similar like Nigerian and Japanese ginger (Owolabi *et al.,* 2007). It has been widely searched that phytochemicals in different plant parts as well as in different varieties of the same species varied significantly (Ghasemzadeh, Jaafar, Rahmat, 2010; Gupta *et al.,* 2011). In addition, methods of extraction used or geographical location also affect the contents of bioactives (Owolabi *et al.,* 2007). The Chinese ginger was found to contain a higher amount of individual phenolic acids in comparison to the Pakistani grown ginger which might be in due part to varied agro climatic conditions of the regions as well as genetic makeup of the varieties tested (Koch *et al.,* 2017).

TABLE I - Profiling of individual phenolics in extracts from Pakistani and Chinese grown ginge

Protocatechuic acid Vanillic acid			Phenolics content (mg/kg)						
			Vanillin	*p*-coumaric acid	Ferulic acid	Sinapinic acid		Total phenolics (mg/kg)	
Solvent extracts	Pakistani grown ginger	EtOH	—	53.40 ±1.23 ^{i}	398.30 ± 1.44 ^{g}	—	110±1.83^{j}	5.09±0.34^{g}	566.79
		MeOH	—	190.6 ±2.64 ^{f}	1019.50±3.14^{e}	71.80±1.34 ^{a}	359.80±1.04^{e}	18.80±1.12^{c}	1660.50
		Ether	49.40±2.22^{a}	78.10 ±1.60 ^{h}	428.70±1.14 ^{e}	45.80±11.83^{b}	198.30±1.65^{g}	16.80±0.14^{d}	817.10
		EtOAc	—	322.20±2.26 ^{d}	2289.20±2.22 ^{c}	31.80±1.36 ^{c}	453.30±1.86^{d}	59.80±1.12^{b}	3156.30
		$CHCl_3$	38.30±1.98 ^{b}	480.40±1.67 ^{a}	1142.10±2.17 ^{d}	52.30±1.87 ^{b}	426.80±2.87^{c}	67.10±1.17^{a}	2207.00
		H_2O	2.06±0.44 ^{c}	142.40±1.82 ^{g}	218.20±1.10 ^{h}	—	11.30±0.80^{k}	12.50±0.40^{e}	386.46
	Chinese ginger	EtOH	1.40±0.27 ^{c}	46.0± 1.69 ^{j}	136.10± 1.19 ^{i}	4.33±0.89 ^{f}	43.30±1.09^{h}	10.40±0.19^{f}	241.53
		MeOH	0.35±0.15 ^{d}	242.20±2.73 ^{e}	405.70±1.11^{f}	26.70±0.90 ^{d}	194.10±1.90^{f}	—	869.05
		Ether	0.26±0.05 ^{d}	378.90±1.71 ^{c}	4305.50±2.11 ^{b}	—	530.70±2.21^{b}	10.20±0.11^{f}	5225.56
		EtOAc	0.06±0.02 ^{d}	5.01±0.72 ^{k}	51.90±1.42 ^{j}	—	36.60±1.72^{i}	—	94.11
		$CHCl_3$	—	486.40±1.93 ^{b}	6338.80±2.87 ^{a}	—	690.20±1.93^{a}	20.50±1.17^{c}	7535.90
		H_2O	0.10±0.04^{d}	6.04±1.74 ^{k}	110.10±1.14^{k}	—	43.08±0.94 ^{h}	4.38±0.14^{g}	163.70

Values are means ± SD (n=3) of three separate experiments. Different superscript letters (^{a,b,c,d,e,f,g,h,i,j,k}) within the same column show significant ($p < 0.05$) differences of means among extraction solvents.

Anti-inflammatory activity

The extracts were analyzed for anti-inflammatory potential; Ibuprofen was used as positive control. EtOAc extract of Pakistani grown ginger showed higher activity on ROS (Table II) followed by methanolic extract of Chinese ginger while the other solvent extracts did not show significant activity at ($p < 0.05$) between two varieties of ginger. A greater activity of ethanolic and methanolic extracts can be linked to higher contents of vanillin in these extracts. According to in-vivo studies, vanillin is well known for its strong anti-inflammatory activity (Niazi *et al.,* 2014). In addition to studied phenolic, there might be other bioactive constituents which might have contributed towards anti-inflammatory potential of these extracts.

TABLE II - Anti-inflammatory activity of extracts from Pakistani grown and Chinese ginger

Extract	Pakistani grown ginger		Chinese ginger	
	% inhibition	IC_{50}±SD	% inhibition	IC_{50}±SD
MeOH	$33.5^{b}{}_{A}$	-	$31.7^{b}{}_{A}$	37.8 ± 2.3
EtOH	$47.1^{a}{}_{A}$	-	$22.4^{c}{}_{B}$	-
CHCl3	$28.8^{c}{}_{A}$	-	$40.3^{a}{}_{B}$	-
Ether	$22.6^{d}{}_{A}$	-	$32.9^{b}{}_{B}$	-
EtOAc	$39.57^{b}A$	26.5 ± 1.8	0.7^{e}	-
H_2O	$26.2^{dc}{}_{A}$	-	$16.7^{d}{}_{B}$	-
Ibuprofen	73.2	11.2 ± 1.9	73.2	11.2 ± 1.9

Values are means ± SD (n=3) of three separate experiments. Different superscript letters (^{a,b,c,d,e}) within the same column show significant ($p < 0.05$) differences of means among extraction solvents. Different caps letter in subscript within the same row and within the same parameter indicate significant differences ($p < 0.05$) between two varieties of ginger (Pakistani and Chinese).

Anticancer activity

Anticancer activity of Pakistani grown ginger and Chinese ginger extracts was determined by MTT assay in comparison with standard drug Doxorubicin (breast cancer drug). The results revealed that most of the tested extracts are active in inhibiting the proliferation of MCF-7 (Michigan Cancer Foundation) cell line (Table III). Water solvent was used to conduct green ecofriendly extracts however, they were found inactive against MCF-7 cell line of both extracts of Pakistani grown ginger and Chinese ginger. The highest inhibition was shown by chloroform extract of Chinese ginger i.e. 91.38 % inhibition as compared to the standard Doxorubicin drug revealing significant ($p < 0.05$) variations among solvents and two ginger varieties. The anti-cancer activity of two ginger varieties was in the order of Chinese ginger > Pakistani grown ginger. Anti-cancer activity of the extracts in the present experiments can be linked to antioxidant/anticancer phenolics in these cases. However, some other anti-cancer agents might have also contributed to bioactivity of these extracts.

According to research reports, anticancer activity of ginger can be linked to the presence of various pungent vallinoids such as (6)-paradol and (6)-gingerol and some other components like zingerone, shogaols (Grzanna, Lindmark, Frondoza, 2005; Shukla, Singh, 2007).

TABLE III - Anticancer activity of extracts from Pakistani grown and Chinese ginger against MCF-7 cell line

Extract	Pakistani grown ginger		Chinese ginger	
	Inhibition	IC_{50}±SD	Inhibition	IC_{50}±SD
MeOH	$74.12\%^{a}{}_{A}$	$18.46 \pm 1.90^{a}{}_{A}$	$55.93\%^{d}{}_{B}$	$38.20 \pm 3.38^{a}{}_{B}$
EtOH	$72.86\%^{a}{}_{A}$	$7.11 \pm 2.30^{c}{}_{A}$	$66.43\%^{c}{}_{B}$	$20.90 \pm 4.3^{b}{}_{B}$
$CHCl_3$	$57.55\%^{c}{}_{A}$	$34.70 \pm 1.28^{b}{}_{A}$	$91.38\%^{a}{}_{B}$	$2.40 \pm 0.09^{d}{}_{B}$
Ether	$64.15\%^{b}{}_{A}$	$21.10 \pm 1.30^{a}{}_{A}$	$81.38\%^{b}{}_{B}$	$4.82 \pm 0.476^{c}{}_{B}$
EtOAc	$45.66\%^{d}{}_{A}$	Inactive	$79.40\%^{b}{}_{B}$	7.06 ± 5.56^{c}

TABLE III - Anticancer activity of extracts from Pakistani grown and Chinese ginger against MCF-7 cell line

Extract	Pakistani grown ginger		Chinese ginger	
	Inhibition	IC_{50}±SD	Inhibition	IC_{50}±SD
H_2O	$29.13\%^{e}{}_{A}$	Inactive	$31.01\%\ ^{e}{}_{A}$	Inactive
Doxorubicin	95%	0.20 ± 0.03	95.00%	0.20 ± 0.03

Values are means ± SD (n=3) of three separate experiments. Different superscript letters (^{a,b,c,d,e}) within the same column show significant ($p < 0.05$) differences of means among extraction solvents. Different caps letter in subscript within the same row and within the same parameter indicate significant differences ($p < 0.05$) between two varieties of ginger (Pakistani and Chinese).

Antimicrobial activity

As ginger is a rich source of condensed tannin (anthocyanidins), so it has been related with anti-inflammatory, antioxidant activity and atherosclerosis prevention. There are different methods for investigation of antimicrobial activity but the most commonly used method is disk diffusion method. Table IV show the antibacterial activity of two varieties of ginger by using six solvents against four types of bacteria. All the tested extracts of both varieties indicate a specific zone of inhibition however; the activity was not the same which might be due to effect of different solvent choice as previously discussed by (Teles *et al.,* 2019).

TABLE IV - Antibacterial activity of extracts from two varieties of ginger

Extract	Zone of inhibition (mm)			
	Escherichia coli	*Bacillus subtilis*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*
MeOH	$12.2\pm0.4^{a}{}_{A}$ (P) $11.2\pm0.11^{a}{}_{B}$ (C)	$15\pm0.00^{a}{}_{A}$ (P) $13\pm0.00^{a}{}_{B}$ (C)	$13.5\pm0.01^{a}{}_{A}$ (P) $13.3\pm0.11^{a}{}_{B}$ (C)	$18.0\pm0.02^{a}{}_{A}$ (P) $14\pm0.01^{a}{}_{B}$ (C)
EtOH	$10.6\pm0.04^{b}{}_{A}$(P) $09.5\pm0.2^{b}{}_{B}$(C)	$14\pm0.01^{b}{}_{A}$ (P) $15\pm0.00^{b}{}_{B}$ (C)	$13.2\pm0.21^{b}{}_{A}$ (P) $13\pm0.33^{b}{}_{B}$ (C)	$12.00\pm0.01^{b}{}_{A}$ (P) $11\pm0.06^{b}{}_{B}$ (C)
$CHCl_3$	$13.8\pm0.01^{c}{}_{A}$(P) $10.2\pm0.03^{c}{}_{B}$(C)	$13\pm0.02^{c}{}_{A}$ (P) $11\pm0.06^{c}{}_{B}$ (C)	$12.5\pm0.04^{c}{}_{A}$ (P) $12.2\pm0.05^{c}{}_{B}$ (C)	$16.00\pm0.02^{c}{}_{A}$ (P) $15.00\pm0.02^{c}{}_{B}$ (C)
Ether	$11.6\pm0.03^{d}{}_{A}$(P) $08.5\pm0.02^{d}{}_{B}$(C)	$14\pm0.00^{d}{}_{A}$ (p) $12\pm0.01^{d}{}_{B}$ (C)	$12.7\pm0.00^{d}{}_{A}$ (P) $13.1\pm0.03^{d}{}_{B}$ (C)	$14.00\pm0.06^{d}{}_{A}$ (P) $7.5\pm0.05^{d}{}_{B}$ (C)
EtOAc	$13.2\pm0.06^{e}{}_{A}$(P) $10.4\pm0.03^{e}{}_{B}$(C)	$13.3\pm0.01^{e}{}_{A}$ (P) $14\pm0.03^{e}{}_{B}$ (C)	$14.5\pm0.00^{e}{}_{A}$ (P) $11.5\pm0.34^{e}{}_{B}$ (C)	$15.00\pm0.04^{e}{}_{A}$ (P) $12.00\pm0.05^{e}{}_{B}$ (C)
H_2O	$7.2\pm0.22^{f}{}_{A}$(P) $8.4\pm0.16^{f}{}_{B}$(C)	$12.2\pm0.0^{f}{}_{A}$ (P) $12\pm.0.01^{f}{}_{B}$ (C)	$12.5\pm0.12^{f}{}_{A}$ (P) $12.1\pm0.00^{f}{}_{B}$ (C)	$10.00\pm.0.01^{f}{}_{A}$ (P) $6.00\pm0.07^{f}{}_{B}$ (C)
Ciprofloxacin	$25\pm0.0^{g}{}_{A}$	$29\pm0.08^{g}{}_{A}$	$20\pm0.0^{g}{}_{A}$	$19\pm0.02^{g}{}_{A}$

Small caps letter (^{a,b,c,d,e,f,g}) denotes significant difference of various solvent with in column while capital letter ($_{A,B}$) describes potential activity against different bacterial strain within rows. All values were taken in triplicate with p value < 0.05. Pakistani grown Ginger (P) and Chinese Ginger (C)

In tested extracts, methanolic extract of Pakistani grown ginger and $CHCl_3$ extract of Chinese ginger showed highest antimicrobial activity against *Pseudomonas aeruginosa* 18.0±0.02 (L) and 15.00±0.02(C) respectively. Minimum results obtained with water solvent for both varieties like Pakistani and Chinese ginger with ranges of 7.2±0.22 (L) and 6±0.07(C) respectively. The higher activities of ethanolic and methanolic are generally referred to polyphenolic enrichment as explained by (Akhtar *et al.,* 2019). The order of solvents for antimicrobial activity against *Escherichia coli* was found to be: methanol> ethanol> ethyl acetate> chloroform> ether> distilled water. Although, the higher activity was the result with Pakistani grown ginger compared to Chinese ginger. The higher antibacterial activity is attributed to higher number of phytochemicals, such as camphene, phellandrene, zingiberene, and zingerone in this species (Mao *et al.,* 2019; Teles *et al.,* 2019). The similar order of samples for antibacterial activity against *Bacillus subtilis* was observed Pakistani grown ginger > Chinese ginger. Against *Klebsiella pneumonia* all tested extract showed significant results. Methanolic and ethanolic extract describe similar results followed as trend methanol= ethanol, ethyl acetate>chloroform= ether> distilled water.

From above results it can be concluded that all solvent extracts of Pakistani grown ginger showed more antibacterial activity against the four tested bacterial strains, as compared to Chinese ginger. Interestingly, both samples showed highest antibacterial activity against the gram-negative bacterial strain *Pseudomonas aeruginosa* contrary to most of the plant extracts in which order of activity is more beneficial towards gram positive strains (Al-Mariri, Safi, 2014). Thus, it shows that extracts of Pakistani grown ginger specially methanolic and ethanolic extracts are most suitable to pose antibacterial activity against the above-mentioned strains of bacteria.

CONCLUSION

The study was conducted to evaluate the differences of anti-inflammatory, anti-cancer activity and phenolic composition between the Pakistani grown and Chinese variety of ginger. The results indicated qualitative as well as quantitative differences of phenolic acids in different extracts of the two varieties. The predominant phenolics in two ginger varieties were found to be vanillin, ferulic acid, p-coumaric acid, sinapinic acid and cinammic acid. The Chinese variety, in general, showed higher phenolic acids content than the Pakistani variety. The anticancer potential of Chinese variety was also higher than that of Pakistani grown ginger; conversely, the Pakistani variety exhibited higher anti-inflammatory activity. With regards to antibacterial analysis extract showed higher activity towards gram-negative strain which shows significant indication of higher amount of certain bioactive having capability to cross additional cell wall layer against these strains. These bioactive needs to be explored and checked for their potential individually in future study. The presence of phenolics along with other bioactive/ anti-inflammatory agents in ginger makes this medicinal herb a promising functional food. Ginger can also be explored for its possible use in different disease models; however further studies are needed to isolate individual bioactive compounds and elucidate their mode of action to develop drugs.

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