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Antibacterial Activity And Phytochemical Analysis Of Ginger (Zingiberofficinale) On Bacteria From Normal Skin Flora

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ABSTRACT

The antibacterial activity and Phytochemical analysis of ginger extracts were studied. Ginger extracts were obtained using, cold water, hot water, ethanolic and essential oil. The extracts were assayed for antibacterial activity. Bacteria were isolated from normal skin flora.The extracts were highly effective against gram positive bacteria . The results also showed that ginger extracts possesses antibacterial properties and could be used for the treatment of bacterial infections. Phytochemical analysis of ethanolic extract of ginger revealed the presence of glycosides, terpenoids, flavonids and phenolic compounds.This medicinal value ginger can be formulated as an drug in the future.

Key words: Antibacterial activity, ginger extracts, Phytochemicals, Microorganism.

INTRODUCTION

Ginger (Zingiberofficinale), Roscoe belonging to the Family Zingiberaceae, is a perennial herb with thick tuberous rhizomes. The erect leafy aerial stem grows up to approximately one meter in height and has purple flowers. Its roots are used as spice in cooking throughout the world. The ginger plant has a long history of cultivation known to originate in China and then spread to India, South East Asia, West Africa and the Caribbean (Weiss, 1997; McGee, 2004). Ginger contains up to 3% of an essential oil that causes the fragrance of the spice (O'Hara et al.,1998). The Zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes (Chen, 2008). Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also "Generally Recognized as Safe" (GRAS).

Ginger is cultivated in the tropics and it requires warm and humid climate flourishing in a well-drained friable soil, though it can also be grown in a light soil rich in humus. It has variety of names from different continents and countries and such names are Zingiberis rhizome, Shenjiany, Cochin, Asia ginger, Africa ginger and Jamaican ginger. Ginger is the underground rhizome of the ginger plant with firm striated texture. The flesh of the ginger rhizome can be yellow, white or red in colour depending upon the variety. It is covered with a brownish skin that may either be thick or thin and it is consumed as delicacy, medicine or spice.(Subramanian et al., 2010)

Ginger is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Ali, 2008). Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections.

The pungent taste of ginger is due to nonvolatile phenylpropanoid – derived compounds, gingerols and shogaols. The shogaols are formed from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process, and it is less pungent and has a sweet aroma (O'Harold, 2004).

Ginger is a minor chemical irritant, and has a sialagogue action, stimulating the production of saliva (O'Hara et al., 1998). Mature ginger roots are fibrous and nearly dry. They can be cooked as an ingredient in many dishes. They can be stewed in boiling water to make ginger tea, to which honey is often added as a sweetener; sliced orange or lemon fruit may also be added. The juice of ginger roots is extremely potent and is often used as spice to flavour dishes such as seafood, mutton, snacks or stew. Powdered dry ginger roots (ginger powder) are typically used to add spiciness to ginger bread and other recipes. Ginger is also made into candy and used as flavoring for cookies, crackers and cakes as well as flavour in carbonated, non-alcoholic beverage, ginger bread, ginger snaps, ginger cake and ginger biscuits (McGee,2004).

Medically ginger is used as a stimulant and carminative, and is used frequently for drypepsia and colic (O'Hara et al., 1998). It has a sialaggogue action, stimulating the production of saliva. It is also used to disguise the taste of medicines. Ginger promotes the release of bile from the gall bladder (Opdyke, 1974; Kato et al, 1993; O'Hara et al, 1998). Ginger may also decrease joint pain from arthritis, may have blood thinning and cholesterol lowering properties and may be useful for the treatment of heart diseases and lungs diseases (Kato et al, 1993; O'Hara et al, 1998).

The characteristic odour and flavour of ginger root is caused by a mixture of gingerone, shoagoles and gingerols, volatile oils that make up about 1-3% of the weight fresh ginger. Gingerols are increasing the gastrointestinal tract's motility, also have anti-bacterial,

sedative, analgesic and antipyretic properties (da SilveiraVasconceloset al, 2019). Ginger has been found effective by multiple studies for treating nausea caused by seasickness, morning sickness and chemotherapy (Ernst and Phittler, 2000). Ginger has been reported to be effective for the treatment of inflammation, rheumatism, cold, heat cramps, and diabetes (Afshari, 2007).

Allergic reactions to ginger include heartburn, bloating, gas, belching and nausea (particularly if taken in powdered form). Unchewed fresh ginger may result in intestinal blockage, and individuals who have had ulcers, inflammatory bowel diseases or blocked intestines may react badly to large quantities of fresh ginger (Opdyke, 1974; O'Hara et al, 1998). Ginger can also adversely affect individuals with gallstones, and may affect blood pressure, clotting, and heart rhythms (O'Hara et al, 1998).

The Minerals are phosphate, chromium, aluminum, cobalt magnesium, amino acids: lysine tryptophane, isoleucine, asparagine, arginine, histidine, leucine, isoleucine ,tyrosine.The essential oils isgama-eudesmol, alfacadinol, alpha-farnesene, beta-bisoloberbisabolol, acid - Alpha linolenic, capric, linoleic, caprylic and ascorbic (Gong et al., 2004).

MATERIAL AND METHODS

Collection and treatment of sample

The fresh ginger rhizomes were collected, cleaned, peeled, sliced and dried at room temperature. After drying, pieces of Zingiberofficinale were grinded to fine particles in isolated manner utilizing a suitable grinder.
Then 100g of the powdered mass obtained was stored in clean sterile bottles at room temperature and used for the extractions.

FIGURE : 1. GINGER

Phytochemical analysis

The preliminary qualitative phytochemical analysis related to ginger's root powder has been carried out for screening the existence of bio-active components in the roots.

Test for tannins

Powdered plant parts (root) related to test plant (1gm) have been weighed in beaker as well as distilled water of (10ml) has been added. The mixture is boiled for 5 minutes, also 2 drops of the 5% FeCl3 have been added. Producing greenish precipitate indicates the existence of tannins.

Test for terpenes

To 0.5gm of the powder 3ml. chloroform have been added as well as filtered, also 10 drops of the acetic anhydride and two drops of the sulphuric acid have been added to filtrate, the change in color from blue to green has been indicated.

Anthroquinones

Plant powder (0.5 gm) has been shaken with benzene (10ml) and filtered , also 5 ml of 10% ammonia has been added into filtrate. The mixture has been shaken, also the existence of violet, red, or pink color specifies the existence of anthroquinones.

Test for saponins

Plant extract (0.5 gm) has been provided in tube which contains distilled water of (5.0ml) to the mixture has been shaken for two minutes, also formation of froth specified the existence of saponins.

Test for flavonoid

5 mL of distilled water and about 0.2 g of plant extract were mixed thoroughly. And 1 mL of 1% AlCl3 solution was added and shaken. A light yellow precipitate indicates the presence of flavonoids.

Test for phenol

About 0.5 g of plant extract was added to 1 mL of 10% FeCl3 solution. A deep bluish green colouration was an indication for the presence of phenol.

Test for ascorbic acid

About 0.5 g of plant extract was added to 2 mL of acetic acid and it was shaken for 3 minutes, and then filtered. Few drops of 2, 6-Dichlorophenolinddophenol solution were added to the filtrate. The presence of faint pink colour confirms that ascorbic acid is present.

Test for reducing sugar

2 mL of distilled water and 0.2 g of plant extract were mixed together and thoroughly shaken in a test tube. 1 mL each of Fehling solution A and B were added to the mixture. A brick-red precipitate at the bottom of the test tube confirms the presence of reducing sugar.

Test for glycoside

0.2 g of plant extract and 2.5 mL of dilute sulphuric acid were mixed together and boiled for 15 minutes, cooled and neutralized with 5 mL each of Fehling solution A and B. The formation of brick red precipitate confirmed glycoside.

EXTRACTION OF GINGER(Mohammed Ibraheem Nader et al., 2010)

Cold water extraction

Exactly 20g of blended ginger rhizomes soaked in 100ml of distilled water for 24 hour. The pulp obtained was left in a clean , sterile glass container and shaken at 150 rpm for 8 hours vigorously to allow for proper extraction.The extraction was filtered using asterile muslin cloth after which the extract was obtained, air dried and stored below ambient temperature until required.

Hot water extraction

Exactly 20g of blended ginger rhizomes were soaked in 100 ml of hot water at 80°C (shaker water bath) at 150 rpm for 24hr.,then the resulted juice was extracted, air dried and stored stored below ambient temperature .

Crude ethanolic extraction

20g of small pieces of fresh ginger rhizomes were soaked in 100ml of 95%ethanol ,and shaken at 150rpm for 24 hours at ambient temperature the mixture then filtered.The filterates were evaporated using vacuum rotary evaporator,and frozen at -20°C . Stock solutions of crude ethanolic extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO)solution.

Essential oils

300g of powdered pieces of fresh ginger rhizomes with distilled water (1L) were placed in flask (2L) together after steam distillation, the essential oils were collected,

dispensed into dark bottles, and stored at 4°C until used .The various extracts were used for the analysis of antibacterial activities and antifungal activities.

Identification of Bacterial Isolates

Bacterial isolates were cultured from normal skin flora using the method of Cheesbrough.Pure cultures of the isolates were obtained by streaking representative isolates on freshly prepared nutrient agar and incubated at 37 for 24hours in the incubator. After incubation, identification was done using the gram staining technique and some biochemical test such as Oxidase, Citrate, Indole, Catalase, sugar fermentation tests and Methyl Red Test.

Assay of antibacterial activity

Antibacterial screening test of extract using disk diffusion method. The disk diffusion test was performed using standard procedure by Jorgensen . The inoculum suspension of each bacterial isolate was swabbed on the entire surface of Muller Hinton agar (MHA)(pH7.3).Sterile 6mm filter paper discs were aseptically placed on MHA surface and crude ethanolic extract, essential oil , hot water extraction and cold water extraction were immediately added to discs in volume of 20 ml. A 20ml aliquot of 10% DMSO and distilled water were also added to a sterile paper discs as a negative control, whereas an antibiotic screening by disc method used as a positive control.The plates were left at ambient temperature for 15 minutes to allow excess predifferent of extraction prior to incubation at 37°C for 24 hr. Diameters of inhibition zone were measured and recorded.

RESULT AND DISCUSSION

Plants are an important source of potential useful bioactive compounds for the development of new therapeutic agents. There are many reports available on the antibacterial, antiviral, antifungal properties of plants. The results of this work indicates that the ginger extracts were more effective on gram positive bacteria (the widest zone of inhibition), but on gram negative it is less effective. This is probably due to the differences in cell wall structure of gram positive bacteriaand gram negative bacteria.(Table : 1)These results agree with observations of the Akoachereet alwho had reported that the extracts of ginger exhibited antibacterial activity against the pathogens Staphylococcusaureus .

In gram negative bacteria it was observed that ginger extracts(except hot water extract) had activity on Esherichiacoli , Salmonella sp and Klebsiella sp. These results are contradictory to the observations of Indueet al, who had reported that the ginger extracts did not show any antibacterial activity against all serogroups of Escherichia coli and Salmonella sp.The ethanolic extract of ginger showed the broadest antibacterial

activity by inhibiting growth of all bacterial isolate tested .The ethanol extraction was being an organic solvent and will dissolve organic compounds better,hence liberate the active component such as zingerone, gingerol and shogaolrequired for antimicrobial activity.It was observed that the cold water extract of ginger was more effective on the bacteria than hot- water extract, this may be explained by the fact that the antimicrobial substances in the ginger extract are destroyed by heat from the hot-water which might have raised the temperature of the extracts inactivating them Nelson et alexplain that the antimicrobial substance in the extract are mainly phenolic compounds were destroyed or inactivated by heat.Ginger essential oil possessed moderate antibacterial activity in this study. The major pungent compound of ginger are gingerone and gingerolwhich have strong inhibitory activity against pathogenic bacteria. Tannins is present in large ,comparatively than ethanol and give dark greenish precipitation. Saponins present in small amounts in the all extraction and same quantity identification formation the froth. Terpenes present equal quantity in the extract give green color has appeared. Anthraquinones is absent in the ginger root extract gives pink, red & violet color (Table 2).

The result of the preliminary phytochemical screening was carried out on the Ethanol extracts of the samples and revealed the presence of a wide range of phytoconstituents including tannins, saponins, flavonoids ,terpenes, reducing sugar as showed in table 2. AskalMaimulyanti revealed that Phytochemical constituents in methanol extract were flavonoids and tannins and in ethylacetate extract was flavonoids, saponin and steroids. This constituent indicated the antioxidant activities in the sample. The presence of these phytochemicals in the extracts of ginger and shows that the extracts have some medicinal and physiological activity suggested by Rahman et al., 2013 .This sample also possess many phytochemicals and also possess medicinal value.

TABLE 1 :Antibacterial activity Of Ginger against Bacteria

ORGANISM	Cold extract	Hot extract	Ethanol extract	Essential Oil
Escherichia coli	14	9	20	11
Pseudomonas aeruginosa	12	8	18	12
Salmonella sp	16	6	22	18

Staphylococcus aureus	18	7	23	20

TABLE 2 : Phytochemical screening of Ginger

Phytochemicals	Ginger Extracts
Tannins	**+**
Terpenes	**+**
Anthroquinones	**-**
Saponins	**+**
Flavonoid	**+**
Phenols	**-**
Ascorbic acid	**+**
Reducing sugar	**+**
Glycoside	**-**

Conclusion

A safe, inexpensive, effective and ecofriendly system is needed to control the human health hazards. The results obtained from antimicrobial activity of ginger extract was that it can be used as an antimicrobial agent in pharmaceuticals. There is wide body of scientific study and evidence to show that ginger has great potential in the treatment of many microbial diseases. The results of our experiments showed that different bacterial species exhibited different sensitivities towards the extract of ginger. Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the plants extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus ginger which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens.

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