

## Antimicrobial Activities and Some Fatty Acids of Turmeric, Ginger Root and Linseed Used in the Treatment of Infectious Diseases

Seher Gur, Dilek Turgut-Balik and Nazmi Gur

Department of Biology, Faculty of Arts and Sciences, University of Firat, 23169 Elazığ, Turkey

**Abstract:** *Rhizoma Curcumae longae* (Turmeric), *Rhizoma zingiberis* (ginger root) and *Semen lini* (linseed) are medicinal plants of which combinations are traditionally used in the treatment of infectious diseases. In this study, methanolic extracts of these plants were assayed for antimicrobial activity against various Gram-negative and Gram-positive bacteria (*Bacillus subtilis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus* sp., *Proteus vulgaris* and *Escherichia coli*). Antimicrobial activity was conducted by the agar well diffusion method. The plant extracts showed various levels of antimicrobial activity on different test microorganisms. The most potent extract was obtained from linseed. But in general, a combination of linseed and ginger root was found to be more effective than they used alone. Turmeric was noneffective on five microorganisms tested. All these results may be, in part, due to the fatty acid content of the plants.

**Key words:** Antimicrobial activity • medicinal plants • agar diffusion method • turmeric • ginger root • linseed

### INTRODUCTION

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to the report of The World Health Organisation, 80% of the world's population rely mainly on traditional therapies which involve the use of plant extracts or their active substances [1]. The microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs [2]. This creates problems in the treatment of infectious diseases [2, 3]. Furthermore, antibiotics are sometimes associated with side effects [4], whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature [5]. All these data highlights the need for new alternative drug regimens.

The antimicrobial properties of the medicinal plants are reported from all over the world recently [2, 6-9] and used in the treatment of many diseases such as, Malaria [10], AIDS and sexually transmitted diseases [5].

In the present study, antimicrobial activities of turmeric, ginger root and linseed were investigated for the aim of discovering the medicinal potential of these plants.

### MATERIALS AND METHODS

**Plant material:** Plant materials were obtained from a local traditional healer in Elazığ, where located at the Eastern Anatolia of Turkey. Roots or seed of the plants were already grounded and sold separately. Traditionally recommended mixing ratios of turmeric, ginger root and linseed are 30.4, 32.4 and 37.05%, respectively.

**Preparation of plant extracts:** Twenty five g of each powdered plant material were soaked with 100 ml of 70% methanol (BDH, UK) for 72 h at room temperature with occasional mixing. Each extract was then filtered with Whatman filter paper number 1, The filtrates were concentrated in vacuo at 30°C and stored at +4°C until further applications.

**Test microorganisms:** The microorganisms used for the antimicrobial activity evaluation were obtained from the Microbiology Laboratory, Department of Biology, Faculty of Arts and Sciences, University of Firat, Elazığ, Turkey. They were; *Proteus vulgaris* FMC 1, *Bacillus subtilis* IMG 22, *Enterobacter aerogenes* CCM 2531, *Escherichia coli* ATCC 25921, *Staphylococcus aureus* COWAN 1, *Klebsiella pneumoniae* FMC 5, *Pseudomonas aeruginosa* DSM 50071, *Listeria monocytogenes* SCOOT A and *Streptococcus* sp.

**Culture medium and inoculum:** The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at +4°C. Cell suspensions were prepared by inoculation of each bacteria into 10 ml of Nutrient buyyon (Difco). Incubation was performed at 37°C for 24 h. On the next day Mueller-Hinton Agar (MHA) (Merck) was prepared and cooled to 45°C. Bacterial suspension was added into MHA to give a final concentration of 10<sup>7</sup> bacteria/ml and plated out.

**Antimicrobial activity assay:** The agar diffusion method was used for the antimicrobial evaluations. Wells of 8 mm diameter were punched into the MHA, having the test microorganism and filled with 50 µl of plant extract. The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the inhibition zone (including 8 mm diameter wells) against the test microorganisms. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8 mm [11].

Standart antibiotic discs Penicillin G (P-10u) and Trimethoprim 1.25 µg<sup>+</sup>, Sulfamethoxazole 23.75 µg (SXT 25) were obtained from the University of Firat, Faculty of Medicine Elazığ, Turkey and used for comparison. Methanol was used as positive control. All of the assays were performed in triplicate and expressed as average values.

**Determination of fatty acids of the samples:** The lipid of samples was extracted by the method of Hara and Radin [12]. Samples were homogenized with the mixture of hexane-isopropanol (3:2, v/v) in MICRA D8 homogenizator. Non-lipid contaminants were removed by washing with 0.88% KCl solution. Aliquots were taken and the total lipid content, the fatty acids esterified with 2% sulfuric acid in methanol and the fatty acid composition determined by gas chromatography [13].

**Fatty acid composition:** Fatty acids in lipid extracts were converted to methyl esters by using 2% sulfuric acid (v/v) in methanol. Fatty acid methyl ester forms (FAME) were extracted with n - hexane. Gas chromatography analysis was employed GC-17A instrument with FID and AOC-20s Autoinjector and Autosampler from Shimadzu (Kyota, Japan). FAMES were separated by fused silica capiller column, 25 m length and 0.25 mm diameter, Permabond (Machhery - Nagel, Germany). Column temperature was programmed between 120-220°C, 5°C/min and the final temperature was hold 15 min. Injector and FID temperatures were 240 and 280°C, respectively. Nitrogen

was used as carrier gas under head pressure of 50 kPa (corresponding to 1.2 ml/min, 43 cm/s column flow rate). Identification of the individual methyl esters was performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions. *Class GC 10* software version 2.01 assisted at workup of the data.

## RESULTS

**Ethnomedical data of the plants:** *Rhizoma Curcumae longae* (Turmeric) is locally called as Zerdeçal. It is traditionally used in the treatment of infectious diseases, hepatitis and liver diseases. This plant increases the bile level and makes stomachaches to disappear. It is also used to stain foods, silk fabrics and tin leathers [14].

*Rhizoma zingiberis* (Ginger root) is named as Zencefil Kökü in public. It is traditionally used to treat infectious diseases and liver diseases. It has yatıştırıcı effect and prevents vomiting, stops diarrhoea. Ginger root is also used as a spice in the foods [14].

*Semen lini* (Linseed) is locally known as Keten Tohumu. This plant is also traditionally used to treat infectious diseases. It regulates the digestive system and effective as purgative. Traditionally, a linseed mesh is prepared and used to decrease pain externally [14].

**Antimicrobial activity assays:** The antimicrobial effect of turmeric, ginger root and linseed against test microorganisms are shown in Table 1. Only the alcoholic extract was tested, as alcohol was found to be better solvent for extraction of antimicrobially active substances compared to water and hexane [2].

According to the results, turmeric extract was not very effective against most of the test microorganisms. Inhibition zone diameters were ranged in between 11.0-11.6 mm.

Ginger root extract was effective against all of the microorganism groups tested. Diameter of the inhibition zones were ranged in between 13.0-19.0 mm.

The extracts of linseed was also showed antimicrobial activity against the microorganism groups tested. Inhibition zone diameters were between 12.3-21.0 mm.

First standart antibiotic disc, used for comparison, was P-10 U. It was effective against all of the microorganism groups except *Enterobacter aerogenes* and *Klebsiella pneumoniae*. Inhibition zone diameters were ranged in between 9.0-16.0 mm.

The second standart antibiotic disc was SXT 25 and inhibition zone diameters around this disc were measured

Table 1: Antimicrobial activities of turmeric, ginger root and linseed methanol extracts on test microorganisms

	<i>Proteus vulgaris</i>	<i>Bacillus subtilis</i>	<i>Enterobacter aerogenes</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Listeria monocytogenes</i> sp.	<i>Streptococcus</i>
Zv	11.000 (0.000)	11.000 (0.000)	-	-	12.000 (1.000)	-	-	11.000 (0.000)	-
Zf	13.000 (1.000)	14.333 (1.154)	14.666 (0.577)	17.333 (1.154)	19.000 (1.732)	16.666 (0.577)	15.333 (0.577)	15.000 (0.000)	18.333 (1.154)
Kt	15.333 (0.577)	16.666 (0.577)	12.333 (0.577)	11.000 (0.000)	18.333 (1.527)	11.666 (0.577)	17.000 (2.000)	12.666 (1.527)	12.666 (0.577)
Zf+Kt	17.333 (0.577)	12.333 (0.577)	13.000 (1.000)	12.666 (0.577)	17.666 (2.081)	13.333 (0.577)	21.000 (1.000)	18.000 (1.000)	12.333 (0.577)
Zv+Zf+Kt	11.333 (0.577)	13.000 (1.000)	12.000 (1.000)	9.666 (0.577)	9.666 (0.577)	12.666 (0.577)	12.333 (0.577)	11.000 (0.000)	11.666 (0.577)
P-10 U	12.000	14.000	-	10.000	16.000	-	15.000	11.000	9.000
SXT 25	-	25.000	26.000	26.000	-	27.000	20.000	-	24.000
Control	-	-	-	-	-	-	-	-	-

(-): No inhibition

Zv: Turmeric, Zf: Ginger root, Kt: Linseed, P-10 U: Penicillin G, SXT 25: Trimethoprim and Sulfamethoxazole, Control: Methanol.

Note: Standart deviations are given in paranthesis. Diameter of the inhibitory zone is in mm.

Table 2: Percentage of the main types of fatty acids identified by GC-MS in ethanol extracts from turmeric, ginger root and linseed samples (%)

Fatty Acids	Turmeric	Ginger root	Linseed
Palmitic acid (16:0)	9.7598	15.4800	6.6906
Stearic acid (18:0)	-	-	3.2858
Oleic acid (18:1)	8.0844	14.1629	18.9675
Linoleic acid (18:2, n-6)	18.6300	15.8904	17.6651
Linolenic acid (18:3, n-3)	6.0062	8.1288	53.3910
Undecanic acid (c 11:0)	-	37.8503	-
Palmitoleic acid (16:1)	32.5034	8.4876	-
C15-10- pentadecenoic acid (15:1)	4.0394	-	-
C15-10- heptadecenoic acid (17:1)	20.9768	-	-

as between 20.0-27.0 mm. SXT 25 was found to be ineffective on *Proteus vulgaris*, *Staphylococcus aureus* and *Listeria monocytogenes*.

Inhibition zone was not observed around any of the control group, ethanol.

**Fatty acid compounds of turmeric, ginger root and linseed:** Percentages of the main types of fatty acids which were identified by GC-MS in ethanol extracts from turmeric, ginger and flaxseed samples. Flaxseed was rich in linolenic acid (53.4%), ginger in undecanic acid (37.85%) and turmeric in palmitoleic acid (32.50%) (Table 2).

## DISCUSSION

Plant originated antimicrobial drugs are of interest because in part many human and animal pathogens show

multi-drug resistance and in part certain antibiotics have undesirable side effects [7]. In this study, ethanol extracts of three plants were prepared and tested for antimicrobial activity against some test microorganisms.

Ginger root and linseed extracts were found to be more effective than turmeric extracts on test microorganisms.

Turmeric extract was not effective against *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus* sp. It was most effective on *Staphylococcus aureus* and had very little effect on *Proteus vulgaris*, *Bacillus subtilis* and *Listeria monocytogenes*. In general, turmeric extract was exhibited the lowest antimicrobial activity as compared to the other plant extracts tested. Methanolic extract of ginger root was most effective on *Staphylococcus aureus*, with producing an inhibition zone of 19.0 mm.

This inhibition zone is the highest zone produced against this bacteria by the plant extracts and standart antibiotics tested in this study. This plant had its lowest effect on *Proteus vulgaris*. Sensitivity of test organisms against methanolic extrat of ginger root was, in increasing order; *Proteus vulgaris*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus* sp., *Staphylococcus aureus*.

Methanolic extract of linseed was also effective on all of the test microorganisms. This plant extract was also most effective on *Staphylococcus aureus*. The antimicrobial effect of linsed extract against test microorganisms was, in increasing order; *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Enterobacter aerogenes*, *Streptococcus* sp., *Proteus vulgaris*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. All of the three plant extracts were found to be the most effective against Gram-positive bacteria *Staphylococcus aureus* which cause inflammation and food poisoning. But when a mixture of the most effective extracts of plants, ginger root and linseed, was used, antimicrobial activity was reduced on the same bacteria.

The combination of ginger root and linseed extract was exhibited better antimicrobial activity than when they were used alone. For example, inhibition zones were higher against *Proteus vulgaris*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*, in increasing order. In contrast, the combination of the three plant extracts showed less antimicrobial activity compared to the results obtained when these plant extracts were used alone (Table 1). Because these three plants are being sold as a mixture to treat infectious diseases traditionally, we conclude that it is very important to note the correlation between the shown antimicrobial activity and claims of traditional healers.

All these differences in the antimicrobial activity of the extracts might be due to the chemical composition of the plants, the species of the microorganisms used and method of extractions.

Among the medicinal plants tested in this study, both ginger root and linseed extracts showed valuable antimicrobial activities. Further studies are needed to find out the active compounds of these plants. We conclude that, it is possible to find better therapies for many infectious diseases from the plant extracts.

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