

A Comparative Study of the Extracts' Effect of Two Types of Tea Plant on Three Types of Bacteria - Isolated from Diarrhea

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Abstract

In this study, the five dilutions of alcoholic and water extracts of *Thea sinensis* and *Hibiscus sabdariffa* were prepared then the inhibitory effect of all extracts of the plants used in the study have also been studied on three of gram negative bacteria isolated from diarrhea. The conclusions have shown that the alcoholic extract from both plants has a higher effect in all microbes compound to the water extract. The alcoholic and water extract of the *Hibiscus sabdariffa* has a higher inhibitory effect from that of *Thea sinensis*. The minimum inhibitory concentration (M. I. C.) has also been identified in this study for the alcoholic and aqueous extract of the plant related to the study. The identification of this concentration was different according to the type of the extract and the microbe.

Introduction :

Diarrhea is considered one of the most important health problems in societies of low economic level. It is considered one of the major and important causes of death in the world among the suckling infants in particular. (3.3) Million death cases have been registered in developing countries (1, 2).

Bacteria are the major causes of diarrhea as they have a direct effect on small and large intestines and cause Gastroenteritis .

The appearance and spread of bacteria resistance towards antibiotics incited many specialists for research to find substitute antibiotic materials against bacteria .

Such substitutes like medical plants, which contain active materials against bacteria and were confirmed by several studies (3) .

Since the current research includes a study on the effect of the extracts of two types of Tea plant on the growth of three various types of bacteria that cause diarrhea; therefore it is necessary to provide a simple description of the two plants under discussion.

Local black tea :(*Thea sinensis*)

A shrubby-growth plant of the *Camellia* class. Its activity lies on its leaves, which contain (1-5%) of caffeine alkaloid as well as Altaneen – a diarrhea-stop material, volatile oil and Amino acids. Local tea plant is useful in treating diarrhea cases and is also considered an anti-cancer material (4,

5, and 6). Studies also showed that local tea activates the memory of the people drink it .(Y)

Hibiscus sabdariffa L. (Rosella):

This plant varies between a shrubby and herbaceous type of the Malvaceae class. Calyxes' foliage and seeds are its medical parts, containing organic acids, natural pigments, and Vitamin C and tannin materials. Its leaves are used in treating Bronchitis and a comfortable of blood pressure and nerves tranquilizer (8, 9) some studies showed the extracts of the *Hibiscus sabdariffa* has deadly effect on Tuberculosis bacillus. The Scientists had found both types of tea (red and green) inhibit the activity of the enzymes the development of Alzheimer Disease.

Materials & Methods of Operation :

Cultures Three types of the gram-negative bacteria and which are isolated from the diarrhea.

Escherichia coli & *Salmonella typhimurium*: taken from the labs of higher studies department. of microbiology college of education.

Shigella shiga: Taken from the lab. Of microbiology Al-Salam hospital in Mosul .Biochemistry tests of each isolation have been performed to confirm its purity after being cultured back in the mediums of each type .

Collecting medical plants:

The used plants have been collected from the local markets of Mosul; then cleaned and kept in special dried conditions in paper envelopes until the preparation of its extracts.

Preparing plant extracts :

Water extract: Water extract has been prepared according to Riise (11) method by mixing the plant with distilled water by (1: 4) (weight: size). The mixture has been crushed into icebath and was moved by an electric motor to explode the cells' walls. The mixture was soaked for (24) hours and filtered by a piece of gauze then filtered by a filtering paper type Whatmann No. 1.

Alcoholic Extract: This extract has been prepared by using Grand (12) method where (20 gr.) of the plant model was melted in (200 cm.³) of Ethile Alcohol with a concentration of 95% into an icebath; then mixed well and left in the refrigerator for 24 hours and filtered through many layers of gauze. Ethanol was dismissed by using a rotary vacuum evaporator. Next, the extracts are dried by cooling under a detached pressure in a lyophilizer device (provided from Edwards company).. Then the samples are well kept by freezing until be used. In order to conduct inhibitory experiments; one gram of the dry extract was taken and melted in (5 cm.³) of distilled

water and was sterilized by passing it through membranous filters with holes (0.22) micron. This extract was considered a standard to prepare later dilutions. As to alcoholic extract; it was prepared by melting (I gr.) of the standard plant extract in (5 cm³) of (Dimethyl Sulfoxide DMSO) and the mixture was sterilized by pasteurization in (62°C) temperature for (15) minutes (II).

Inhibitory activity of the prepared plant extracts was tested by the sensitivity test method; following the method of Bauer et al (13). Filtering pieces (discs) of paper type (Whatmann No.1) diameter of (6 ml.), sterilized and saturated with the following concentrations (12.5, 25 / 50 / 100, 200 ml./cm.³) of each of the prepared plant extracts; then (0.1cm³) of each of the plant extract concentration was added to a bottle containing (10) sterilized discs (14, 15). The discs were fixed by a sterilized pair of tongs on the surface of nutrient agar medium which was injected by the bacteria under discussion. Plates were incubated in temperature of (37C) for (14-16 hours). In order to show sensitivity of the used extracts; the method of Prescott et al (16) which depends on the inhibitory belt has been followed. Antibiotic; Tetracycline and Earthromycin have been used as positive controlling samples for the bacteria under discussion.

Turbidity method was also used by adding (0.1 cm³) of the plant extract (with different concentrations) to tubes containing (9,8 cm.³) of Nutrient Broth. The tubes were injected by (0.1 cm.³) of the bacterial suspension with a concentration of (10⁸) MI / cm³.

Each concentration was purified three times; then the tubes were incubated in optimum conditions and turbidity was measured by spectrophotometer at a wavelength of (597) manometer and effect activity was measured compared to the controlling samples.

Determination of minimum inhibitory concentration:

These concentrations have been determined by using turbidity test. The following dilutions of each plant extract were prepared (12.5, 25, 50, 100, 200 mlg \.cm³). The minimum inhibitory concentration which is the highest dilution that inhibits the growth of bacteria compared with the controlling sample that consists of (9.8 cm³) from the nutrient broth injected with (0.1cm³) of the bacteria suspension and (0.1 cm³) of each of the plant extract.

Statistical Analysis :

Data were statistically analyzed by choosing significant differences between mediums as well as using Dunckan multi-ranges test by operating SPSS (statistical package for social sciences) program – windows 1999 and a computer Pentium III.

Findings & Discussion :

In this study the inhibitory effect of water and alcoholic extracts of *Thea sinensis* (black tea) and *Hibiscus sabdariffa* L. (Rosella) in the growth of the used bacteria which are widely spread and cause a lot of pathologic cases for human; diarrhea in particular has been determined. The used extracts under discussion have shown an anti-effect on the growth of diarrhea-isolated bacteria and it was also noted that this effect depends on the type of the extract and its concentration. The resistance and spread of serotypes due to the nature of the carriers disease, treatment maner and the pestilence. Leminor1984 emphasized that the efficacy of this factors in diffusion and develop improve certain species of germs sequencly(17)

Table No. (1) explains the effect of the used extracts under discussion on the growth of bacteria *S. typhimurium* and *E. coli Sh. shiga* isolated from diarrhea compared with the two antibiotics Tetracycline & Earthromycin. All the extracts had shown a good inhibitory action compared to the two antibiotics other than the water extract of the *Thea sinensis* (the black tea) which had no inhibitory effect on *Sh. shiga* bacteria. The reason might be that this type of bacteria has many factors of predation and make it resistant for the extracts.

Table No (٧).shows that alcohol extract of *Thea sinensis* has a good anti action for the growth of the both bacteria ;*S. typhimurium* and *E. coli*, and its effect has been related directly which the increase of concentration. There were significant differences at the level ($p < 0.05$) between the averages of inhibition diameters and the effect of each concentration on both bacteria. It has no effect on *Sh. shiga* bacteria in the high concentrations compared to the low concentrations of the extract. The reason is that the cell wall of the bacteria is a nutrient Penetrative of low concentrations with significant differences between inhibitory diameter averages so this compounds of extract have inhibitory effect, and we can explane this efficacy either from their effect on the cell wall or from their relationship with enzymes, DNA, ribosomes, sytoplasmic membrane and other part of the germ cell.(18)

The water extract of the same plant had shown a less effect than alcohol extract as is shown in Table No. (3); the reason is that its active materials have a higher melting capacity in alcohol than it is in water. This result has agreed with what had been submitted by the researcher Al-tae (19) significant differences had been shown between inhibition diameter averages and the effect of each concentration.

All the used bacterial isolations under discussion showed a response to alcohol extract of the (Rosella) as explained in table No. (4). It had a very high inhibitory action compared to other used extractions in the study. The concentration (200ml \ cm³) had shown a higher inhibitory action than the other concentrations of the extract. The reason is that the effect of this extract relates directly with the increase of concentration; and this result agrees with what researcher Al-jarjary had obtained (20).

Table No. (5) shows that the extract of (Rosella) had a less inhibitory effect in the growth of both bacteria *S. typhimurium* and *E. coli* than alcohol extract of the same plant .The table has also shown that this extract has an anti-effect towards *Sh. shiga* bacteria with the increase of the extract concentration. The reason is attributed to the increase of wall penetration of the extract nutrient material. Significant differences between the mediums were estimated by ($P<0.05$) as been shown in table No. 5.

When a comparison was made between the effects of both plants in the bacteria under discussion; we found -generally- that the effect of the Rosella extract was higher than that of the black tea. The reason could be attributed to the nature of the active materials of the Rosella which has an anti-action on the growth of bacteria by containing a high percentage of organic acids such as Malice acid, Sitrice, tartarice and Escorbice which are not found in the black tea and are the cause of the active inhibition effect on bacteria.

Prepared plant extracts on the three bacterial isolation have been tested and their (MIC) were estimated by preparing the previously mentioned concentrations ;where (0.1 cm^3) of each concentration was added to bottles containing 9.8 cm^3 from the nutrient broth medium injected by (0.1 cm^3) of the bacterial suspension; therefore the extract concentration be as follows: (0.125, 0.25, 0.5, 1.0, 2.0) m./ cm^3 respectively.(M.I.C)findings varied according to the type and concentration of the extract as well as the type of the used bacteria.

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Table No. (1): The inhibitory activity of water and alcohol plant extracts in three types of bacteria isolated diarrhea (inhibition circle diameter is measured by millimeter).

Type of Processing	Bacteria Type		
	<i>E. coli</i>	<i>Sh. Shiga</i>	<i>S. typhimurium</i>
Alcohol extract of black Tea	a 0.58 ± 13	Aa 1.16±21	aa 1.0 ± 20
Water extract of black tea	a 1.0 ± 12	...	a 0.58 ± 16
Alcohol extract of the Rosella	a 0.0 ± 17	Aa 1.16± 16	aa 0.58 ± 22
Water extract of the Rosella	a 1.0 ± 13	A 1.1 ± 21	a 0.58 ± 10
Tetracyclin	...	19	20
Earethromycin	...	10	12

* Diameter average of inhibition circle by using three isolations for each sample.

*(...) Refers to the non -existence of an inhibitory activity.

*Numbers after the mark ± represent the trial error .

* Disc's diameter is (6ml).

*letters (a,aa) mean statistical level.

Table No. (2): The effect of alcohol extract of *Thea sinensis* (black tea) with different concentrations on three types of pathologic negative gram stain bacteria (inhibitory circle diameter is measured by millimeter)

Concentration	Bacteria Type		
	<i>E. coli</i>	<i>Sh. shiga</i>	<i>S. typhimurium</i>
200	a 0.58 ± 13	C 0.0 ± 0	aa 1.0 ± 20
100	aa 1.0 ± 11	Cc 0.0 ± 0	a 0.58 ± 17
50	aa 0.5 ± 8 ^a	aa 1.16 ± 21	bb 0.0 ± 13
25	b 0.0 ± 0	Ab 1.0 ± 18	b 1.0 ± 11
12.5	c 0.0 ± 0	B 0.0 ± 16	c 0.0 ± 0

* Diameter average of the inhibitory circle by using three isolations for each sample.

*Vertically different letters indicate a significant difference at the significant level (p < 0.05) .

*letters (a,aa,ab,b,c,cc) means statistical levels.

Table No. (3): The effect of the water extract of *Thea sinensis* (Black tea) with different concentrations on three types of pathologic negative gram stain bacteria (inhibitory cycle diameter is measured by millimeter)

Concentration	Bacteria Type		
	<i>E. coli</i>	<i>Sh. Shiga</i>	<i>S. typhimurium</i>
۲۰۰	a 1.0 ± ۱۲	a 0.0 ± 0	a 0.58 ± ۱۶
۱۰۰	b 1.16 ± ۹	b 0.0 ± 0	b 1.0 ± ۱۳
۵۰	cc 0.0 ± ۰	C 0.0 ± ۰	c 0.0 ± ۹
۲۵	c 0.0 ± ۰	d 0.0 ± 0	dd 0.0 ± ۰
۱۲.۵	cc 0.0 ± ۰	e 0.0 ± 0	d 0.0 ± ۰

*Diameter average of the inhibitory circle by using three isolations for each sample .

*Vertically different letters indicate significant differences at the significant level (P < 0.05).

*letters (a,aa,b,c,cc,d,dd) means statistical levels.

Table No. (4): The effect of alcohol of the Rosella with different concentration in three of pathologic negative gram stain bacteria (inhibition circle diameter is measured by the millimeter)

Concentration	Bacteria Type		
	<i>E. coli</i>	<i>Sh. Shiga</i>	<i>S. typhimurium</i>
۲۰۰	b 0.58 ± 15	A 1.16 ± 16	aa 0.50 ± ۲۲
۱۰۰	A 0.0 ± ۱۷	B 1.0 ± ۱۲	a 0.58 ± ۱۹
۵۰	c 1.0 ± ۱۳	c 1.0 ± ۸	Bb 1.0 ± ۱۵
۲۵	Dd 1.0 ± ۱۰	Dd 0.0 ± ۰	B 0.0 ± ۱۳
۱۲.۵	D 0.58 ± ۹	d 0.0 ± ۰	c 0.0 ± ۰

*Diameter average of the inhibition circle by using isolation for each sample.

*Vertically different letters show a significant difference at the level of significant (P < 0.05) .

*letters (a,aa,b,bb,c,d,dd) means statistical levels.

Table No. (5) : The effect of water extract of the Rosella with different concentrations in three types of pathologic negative gram stain bacteria (inhibition circle diameter is measured by the millimeter)

Concentration	Bacteria Type		
	<i>E. coli</i>	<i>Sh. Shiga</i>	<i>S. typhimurium</i>
٢٠٠	a 1.0 + ١٣	a 1.16 + ٢١	a 0.58 + ١٥
١٠٠	b 0.0 ± 0	bb 1.0 + ١٥	b 1.16 + ١٢
٥٠	c 0.0 + ٠	bb 0.58 + ١٤	cc 0.0 + ٠
٢٥	d 0.0 + ٠	bb 1.0 + ١١	cc 0.0 + ٠
١٢.٥	e 0.0 + ٠	b 1.16 + ٩	c 0.0 + ٠

- * Diameter average of the inhibition circle by using three isolation for each sample .
- *Vertically different letters show significant difference at the level of significance (P <0.05).
- *letters (a,b,bb,c,cc,d,e) means statistical levels.

Table No. (6): The Minimum inhibitory concentration (MTC) of different types of active plant extracts under discussion in three types of pathologic negative gram stain bacteria isolated from diarrhea .

Concentration	Bacteria Type		
	<i>E. coli</i>	<i>Sh. shiga</i>	<i>S. typhimurium</i>
Alcohol extract of black Tea	٠.٥	٠.١٢٥	٠.٢٥
Water extract of black tea	١.٠	...	٠.٥
Alcohol extract of the Rosella	٠.١٢٥	٠.٥	٥.٢٥
Water extract of the Rosella	٢.٠	٠.١٢٥	١.٠

*(...)Refers to non- existence of inhibitory activity.

دراسة مقارنة لتأثير مستخلصات كحولية لنوعين من نبات الشاي على ثلاثة أنواع من البكتريا

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الخلاصة

تضمنت الدراسة تحضير خمسة تخافيف من المستخلصات الكحولية والمائية لكل من نباتي الشاي الأسود والأحمر ودراسة تأثيرهما التثبيطي في نمو ثلاث أنواع من الجراثيم السالبة لصبغة كرام المعزولة من الإسهال. أظهرت النتائج أن للمستخلص الكحولي لكلا النباتين فعلاً تثبيطياً عالياً مقارنة بالمستخلص المائي، كما كان لمستخلص نبات الشاي الأحمر (الكحولي) تأثيراً تثبيطياً أعلى من

مستخلص نبات الشاي الأسود. حدد التركيز الأدنى المثبط (Minimum Inhibitory Concentration) (M. I. C) للمستخلصات الكحولية والمائية للنباتين قيد الدراسة وقد تبين تحديد هذا التركيز حسب نوع المستخلص ونوع الجرثومة.