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ANTI-MICROBIAL ACTIVITY OF *CINNAMOMUM CASSIA* AGAINST DIVERSE MICROBIAL FLORA WITH ITS NUTRITIONAL AND MEDICINAL IMPACTS

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Abstract

Using standard disk diffusion method the antibacterial activity of aqueous infusion, decoction and essential oil of *Cinnamomum cassia* (Cinnamon bark) were investigated against 178 bacterial strains belonging to 12 different genera of bacterial population isolated from oral cavity of 250 specimens of apparently healthy individuals aged between 2-85 years. Overall, the oil of *Cinnamomum cassia* inhibited all type of tested bacterial strains except *Salmonella para typhi* B exhibiting 99.4% antibacterial effect as compared to aqueous decoction (70.2%) and aqueous infusion (52.2%).

Introduction

In the plant kingdom, almost all plants are medicinal and the application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession. In the developing countries, drugs are not only expensive but also have many side effects during treatment for any disorders that is why now in this era it is being emphasized to search medicinally valueable plants and predict their biological activity. Scientific evidence is accumulating and many of the plants have medicinal properties that alleviate symptoms or prevent diseases (Lai & Rov, 2004) eg., diarrhoea, asthma, urinary tract infections (UTI), septicemia, neonatal-meningitis, typhoid fever, osteomyelitis, gasteroenteritis, bone/lung abscesses, food poisoning, sickle cell disease, shigellosis, Reiter's syndrom, dental disorders etc. The characteristics of the plants that inhibit microorganisms and are important for human health have been researched in laboratories since 1926 (Erdogrul, 2002). Traditional medical treatments in daily life are now being used with empiric methods. Research interest has focused on Cinnamon that possess chemopreventive, antispasmodic, anti-ulcer, choleretic, sedative, hypothermic, antifungal, antibacterial, antiviral, antipyretic, lipolytic, antiseptic, anesthetic, anodyne, cytotoxic, hypolipidemic, antiplatelet properties and also stimulate immune system that may be useful adjuncts in helping to reduce the risk of cardiovascular disease and cancer (Cralg, 1999). Cinnamon has been a favorite spice around the world not only because of its health benefits but also because it flavors and preserves food. Cinnamon is native of Southern Asia and South America. It is also now cultivated in many tropical countries such as India, China, Madagascar, Brazil, Mexico and the Caribbean. Cinnamon (*Cinnamomum cassia*) of the family Lauracease is also known as Sweet wood and Gui Zhi. It contains medicinally important essential oil in leaves, fruits inner and outer bark. Much of cinnamon's bioactivity resides in its oil, which is about 90% cinnamaldehyde. It is used mainly in medicine, foods and cosmetics (Bown, 1995), and is employed in aromatherapy as a rub to promote blood circulation. It also contains both anti-fungal and anti-bacterial principles that can be used to prevent food spoilage due to bacterial contamination (Fabio *et al.*, 2003).

The fruits of cinnamon (Cassia Buds) resemble cloves in appearance and are widely used for flavoring in the food industry. Cinnamon is also used as an invigorating tonic. The constituents of cinnamon are volatile oil (Cinnamaldehyde, eugenol, Cinnamic acid, weitherhin), mucilage, diterpenes, proanthocyanidins (Jayaprakasha *et al*., 2002) and posses antioxidant actions which may prove beneficial against free radical damage to cell membranes (Dragland *et al.,* 2003). Eugenol exhibits the antiseptic, anesthetic, anodyne and cytotoxic effects. Cinnamaldehyde and cinnamon oil vapors are potent anti-fungal compounds. Preliminary human evidence confirms this effect in studies of AIDS patients with oral candida (thrush) infections that improve with application of cinnamon oil. Research indicates that cinnamon oil kill mosquito better than DEET. Medicinally, cinnamon is used in western medicine, mainly in treatment for diarrhoea (Skidmore-Roth, 2003), flatulent dyspesia, colic and colds, poor appetite, low vitality, kidney weakness, rheumatism and coldness. It is also used in influenza, cough, bronchitis (Martinez, 1989), fevers, arthritic angina, and palpitations, stimulates the circulatory system and capillary circulation, spasms, vomiting, controls infections, gastric ulcer and digestive or stomach complaints related to cold and chills. Cinnamon is externally used as a skin antiseptic to treat minor bacterial and fungal infections of the skin (Aguilar, 1999) and promote a rosy complexion in face that could increase skin beauty. It is sometimes used alternately with Damiana (*Turnera diffusa* Wild) to promote conception (Adame & Adame, 2000) and for the treatment of hypertension and nervous disorders. Some of the plant constituents have shown effects against fungi, including the molds that produce the carcinogenic aflatoxins and 80% bacterial activity has been found (McCann, 2003). Even though cinnamon has anti-bacterial effects, clinical trials against *Helicobacter pylori*, associated with gastric ulcer (Martin & Ernst, 2003), have shown contradictory results. It is proven to be particularly effective against some species of toxicogenic fungi as well as respiratory tract pathogens, including species belonging to the genera *Aspergillus*, *Candida*, *Cryptococcus* and *Histoplasma* (Inouye *et al*., 2002). Cinnamon significantly reduces blood sugar levels in diabetics. The discovery was initially made accidentally by Richard Anderson at the US Department of Agriculture's Human Nutrition Research Center in Beltsville, Maryland (Boradhurst *et al*., 2000). Researchers at the University of Illinois at Chicago have discovered that cinnamon flavored chewing gum exhibits strong antibacterial effect in the mouth and even counters bad breath. The common name Cinnamon encompasses many varieties, including *Cinnamomum cassia*, *C. camphora* and *Cinnamomum saigonicum*, which are used interchangeably with *zeylanicum*. Using disk diffusion method (Andrews, 2004) the antimicrobial effect of aqueous infusion, decoction and essential oil of *Cinnamomum cassia* were investigated against 200 bacterial strains belonging to 15 different genera of bacterial population isolated from oral cavity of 250 specimens of apparently healthy individuals, aged between 2-85 years.

Materials and Method

Oral specimens were collected by gentle rubbing of the sterilized cotton swabs to teeth, gums, tongue and palate. The swabs with specimens were inoculated on Mac-conkey agar (for Gram negative bacteria), tryptic soya agar (TSA) medium and sodium azide blood agar (for Gram positive bacteria) by rolling the swabs over the area of primary inoculation and then streaking was performed with sterile wireloop by clock streak method (Sonnenwirth & Jarett, 1980). The bacterial isolates were identified by morphological, cultural and biochemical methods following standard procedures (Facklam, 2000).

Cinnamon bark and its oil used in this study were obtained from retail market Malir Cantt., Karachi, Pakistan. Decoction of Cinnamon was prepared by boiling 10g ground cinnamon bark in 100ml sterile distilled water in a flask for 20 minutes. The flask containing ground bark of cinnamon and decoction was removed from the heat and allowed to cool. The content of flask was filtered through filter paper to obtain clear decoction. The aqueous infusion was prepared by taking 10g ground cinnamon bark in 100ml sterile distilled water, left for 48h at room temperature and filtered to obtain clear infusion. Sterilized discs of filter paper (6 mm diameter) were soaked in 1ml of infusion, decoction and oil of bark of cinnamon for 1-2minutes and then used for screening. Thus, potency of each disc was 10µl. Mueller-Hinton agar (MHA) (Merck) was used as antimicrobial susceptibility test medium and Mueller-Hinton broth (MHB) (Merck) was used for preparation of inoculum. A sterile inoculating loop was touched to four-five isolated colonies of the tested bacterial strains growing on agar and then used to inoculate a tube of culture broth (Mueller-Hinton). The culture was incubated for 24h at 37°C until it became slightly turbid and was diluted to match a 0.5 McFarland turbidity standard. A sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of a MHA plates. After the agar surface had dried for about 5 minutes previously soaked disc with aqueous infusion, decoction and oil of *Cinnamomum cassia* respectively were placed on it with sterile forceps. All treated plates were immediately placed in a 37°C incubator. After 24 h of incubation the diameter of zone of inhibition were measured and recorded in mm.

Result and Discussion

Of the 178 bacterial strains belonging to 12 different genera of gram positive and gram-negative microbial flora were used in the present study. The result of *in vitro* antibacterial activities of the aqueous infusion, aqueous decoction, and oil of *Cinnamomum cassia* (Cinnamon bark) is given in Table 1 and Fig. 1. The zones recorded included the size of filter paper disc (6 mm in diameter) and potency for each disc was 10µl.

The results showed that the essential oil of *Cinnamomum cassia* posses great antimicrobial properties (99.4%) than aqueous decoction (70.2%) and infusion (52.2%) because of cinnamaldehyde is major volatile and divers constituent present in it and also has variety of active components viz., eugenol, cinnamic acid, weitherhin, mucilage, diterpenes, proanthocyanidins. These active constituents posses both anti-fungal and anti-bacterial properties that could be used as a medicine to prevent the human health effecting disorders. (Bown, 1995). Previous literature has reported that cinnamaldehyde kills 80% bacteria and fungi (McCann, 2003). In the present study, essential oil of *Cinnamomum cassia* inhibited 99.4% of the organisms but was not found effective against *Salmonella para typhi* B. The oil was found highly effective against *Streptococcus oralis*, *Streptococcus anginosus*, *Streptococcus intermedius* and *Streptococcus sanguis*, *Enterobacter aerogenes* and *Micrococcus roseus*. These results are similar with the reports of Ates *et al*., (2003) about *Cinnamomum cassia* who observed antimicrobial effect of essential oil of cinnamon and found remarkable inhibition against variety of tested bacterial and fungal strains.

Table 1. Antibacterial activities of aqueous infusion, aqueous decoction and oil of *Cinnamomum cassia* (Cinnamon bark) against microbial flora isolated from human oral cavity.

Organisms	No. of isolates	Average diameter of zone of inhibition*		
		Aqueous infusion	Aqueous decoction	Oil
Aeromonas hydrophila	2	0	8	12
Alcaligenes sp.	4	0	0	11
Citrobacter sp.	3	0	0	6
Enterobacter aerogenes	2	0	0	26
Escherichia coli	25	0	2	5
Flavobacterium sp.	8	2	4	13
Klebsiella ozaenae	16	0	0	2
Klebsiella pneumoniae	9	4	7	12
Micrococcus roseus	3	15	21	25
Plesiomonas shigelloides	3	0	0	6
Pseudomonas aeruginosa	19	0	0	3
Salmonella typhi	5	0	10	16
Salmonella para typhi A	1	0	0	10
Salmonella para typhi B	1	0	0	0
Staphylococcus aureus	2	0	0	9
Streptococcus anginosus	11	13	21	29
Streptococcus intermedius	7	4	20	27
Streptococcus mitis	4	0	10	15
Streptococcus morbillorium	8	3	12	18
Streptococcus mutans	10	5	17	19
Streptococcus oralis	7	10	23	32
Streptococcus salivarius	9	5	16	19
Streptococcus sanguis	10	11	23	27
Streptococcus uberis	9	2	6	15

*Including the diameter of filter paper Disc-6mm

The aqueous decoction of *Cinnamomum cassia* showed high antibacterial activity against *Streptococcus oralis* and *Streptococcus sanguis* (23mm), *Micrococcus roseus* (21mm), *Streptococcus intermedius* (20mm) and *Streptococcus mutans* (17mm), while *Pseudomonas aeruginosa*, *Salmonella typhi para A*, *Salmonella typhi para* B, *Klebsiella ozaenae*, Citrobacter sp., *Enterobacter aerogenes*, *Staphylococcus aureus*, *Plesiomonas shigelloides* and *Alcaligens* sp., were not inhibited. Biavati *et al*., (1996) also studied the anitmicrobial effects of different plants extracts and their essential oil and found good inhibitory effect against gram positive and gram-negative microbial strains. It is interesting to note that aqueous infusion of *Cinnamomum cassia* did not inhibit a number of species of tested microbial strains except *Micrococcus roseus*, *Streptococcus anginosus*, *Streptococcus sanguis*, *Streptococcus oralis*, *Streptococcus mutans*, *Streptococcus salivarius*, *Klebsiella pneumoniae*, *Streptococcus intermedius*, *Streptococcus morbillorum*, *Flavobacterium* sp., and *Streptococcus uberis* which were inhibited (Table 1).

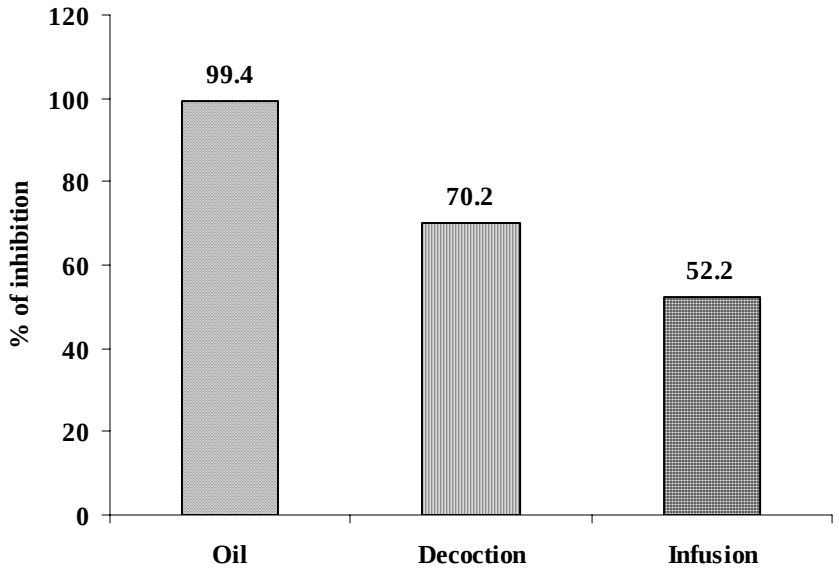

Fig. 1. Comparison of antimicrobial activity between oil, aqueous decoction and aqueous infusion of *Cinnamomum cassia*.

Results reported in the present study contribute to the knowledge of the anti-microbial effect of *Cinnamomum cassia*, as well as confirm their potential application in the treatment and prevention of diseases caused by bacterial flora. Therefore, this study represents an inexpensive or cost effective source of natural mixtures of antibacterial compounds that exhibit potentials for use in food systems to prevent the food borne bacteria and extend the shelf life of the processed food. In addition, it may be effective in reducing the number or preventing the growth of pathogenic bacterial flora eg., *Staphylococcus aureus, Klebsiella pneumoniae,* Salmonella species, *Escherichia coli* and *Pseudomonas aeruginosa* etc.

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