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Antimicrobial activity of extracts of dried kokum (*Garcinia indica* C)

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Abstract: *Garcinia indica* C or commonly known as Kokum and is widely distributed in coastal India. The fruit is mainly found during the summer months, and the rind of the fruit is salted and dried and preserved for use in other parts of the year when it is not available fresh. The rind possesses an important phenolic compound called as Garcinol. The importance of this compound is well known. This investigation, therefore does not aim to study this compound. There are many other compounds which are present, beside garcinol, in the fruit and this study aims to investigate the antimicrobial properties of such compounds. The compounds are furfural and its derivatives, cyanidin- 3-glucose which is present as anthocyanin in the rind and caffeine. Of all these, furfural and cyanidin -3-glucose are potent antimicrobials. The extent to which these compounds get extracted in different solvents determine the degree of bacteriocidal action.

Keywords: Garcinia, furfural, cyanidin, antibacterials, Kokum

Introduction

Garcinia indica C or commonly known as Kokum and is distributed mainly in peninsular India. This is one of several species of Garcinia found in many tropical regions. The Kokum is from a tall tropical evergreen tree (Chandran, 2005). The fruit is harvested during April-May of every year. It is used as culinary in several cooking practices. The extract of the fruit has both antifungal (Selvi *et al.*, 2003) and antibacterial properties and therefore, has a potential for use as biopreservative in food applications (Varalakshmi *et al.*, 2010). The juice has a distinctive acidic flavor. It is a soothing drink in summer months and it provides relief from gastric disorders. It is traditionally used to treat sores, skin ailments such as rashes caused by allergies, dermatitis and chaffed skin, burns, scalds, and to relieve sunstroke. It is also a remedy for diarrhea, dysentery, piles and tumors. It facilitates digestion, purifies the blood and fights cholesterol (Mishra *et al.*, 2006).

It has been found that rind of the fruit contains hydroxy citric acid [HCA], garcinol and the coloring pigment anthocyanin. HCA, which is claimed to have fat-reducing properties, is often used to reduce obesity (Lopes, 2007), since it inhibits the enzyme, citrate lyase responsible for conversion of carbohydrates into fats (Watson *et al*., 1969).

Another major compound reported to be present in the chloroform extract of the fruit is garcinol with a strong antioxidant activity since it contains both phenolic hydroxyl groups as well as a β-diketone moiety (Padhye *et al.*, 2009) and it exerts an anti-inflammatory effect (Liao *et al*., 2005). It acts as a free radical scavenger and hence is very important pharmaceutically (Yamaguchi *et al.*, 2000). This study focuses to characterize the active compounds responsible for antibacterial activity of *Garcinia indica* against strains of bacteria, which cause digestive tract disorders and mild skin infections.

Materials and Methods

Plant material

Since kokum is a seasonal fruit, the rinds of kokum fruits are usually preserved by salting and sun drying. These preserved rinds were collected and homogenized. The ingredients were extracted by using different solvents like water, methanol, ethanol and acetone. The extracts were coarse filtered and later filter sterilized with the help of membrane filters of 0.2µm pore size. The extracts were stored at 10˚C for further use.

Determination of salt content by Mohr's method (Skoog et al., 1996; Sheen et al., 1938 and Kraemer et al., 1924)

The reagents used were NaCl, $CaCO_3$, $NaHCO_3$, 5 % K_2CrO_4 and 0.1M $AgNO_3$.

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Determination of Cl^- in solid sample

The juice of the fruit (70 ml) was extracted with chloroform (100 ml). Since the Chloroform layer was highly colored, distilled water was added to this in 10 fold proportion. This was titrated with silver nitrate solution using potassium chromate as indicator.

Microorganism used

Micrococcus aureus (NCIM 5121), *Bacillus megaterium* (NCIM 2087), *Micrococcus luteus* (NCIM 2103), *Escherichia coli* (NCIM 2066), *Salmonella typhimurium* (NCIM 2501), *Pseudomonas aeruginosa* (NCIM 2036).

The bacteria were grown on nutrient agar (1% peptone, 1% yeast extract, 0.5% NaCl, 2.5 % Agar) at 25°C for 24 hrs.

Antimicrobial activity

The agar well diffusion method was employed. Nutrient agar was used for both Gram positive and Gram negative bacteria. The cooled molten medium was seeded with respective organism. On solidification of the medium, 5mm and 8 mm wells were prepared and filled with 30 µl, 50 µl, 100 µl of extract prepared with different solvents along with pure solvent controls. The tests were carried out in triplicates. The plates were incubated at 25°C for 24 hrs. The zone of inhibition was measured and recorded after subtracting that obtained from corresponding pure solvent.

Determination of active compounds

The methanolic extract of dried kokum was used to determine active compounds in it by Gas Chromatography Mass Spectroscopy (GCMS).

Statistical analysis

Results obtained were the mean of three or more determinants. Analysis of the variants was carried out on all data at $P < 0.05$ using Graph pad software. (Graph pad Instat version 3.00, Graph pad software, San Diego, CA, USA).

Result and Discussion

Determination of salt content

The mean burette reading $AgNO_3$ solution was 0.8333 ml

Standardized molarity of $AgNO_3$ solution = 1.002 ± 0.001 M

Determination of chloride in unknown

Atomic mass of Cl^- = 35.45g/mole.

milli moles of Cl^- = M $AgNO_3$ X V $AgNO_3$

= 0.1002 mmoles/mL X (0.833-0.20) mL

= 0.0634 mmoles

Mass of Cl^- = 0.0634 mmoles X 35.45 mg Cl^- / 1 mmole

= 2.25mg

% Cl^- = mass of Cl^- /mg of sample X 100

% Cl^- = 2.25/10000 X 100

= 0.000225 X 100

= 0.0225

% Cl^- in unknown = 0.0225 ± 0.1

Therefore, %NaCl dried kokum = 0.0370 w/w

It can be seen in Figure 1, that all the extracts of 30 µl volume do not show the same degree of anti microbial activity. *Micrococcus aureus* for example shows a 7 mm zone of inhibition with water extract, but in presence of ethanolic extract the zone of inhibition is 10 mm. On the other hand *E. coli* is not inhibited by any extracts except the ethanolic extract. So also is the case with *S. typhimurium.*

All the extracts of 50 µl volume show different antibacterial activities (Figure 2). *Bacillus megaterium* shows 9 mm zone of inhibition in water extract, 8 mm zone of inhibition in ethanolic extract, 10 mm zone of inhibition in methanolic extract. Whereas *Salmonella typhimurium* shows maximum zone of inhibition in water extract which is not observed for 30 µl of water extract.

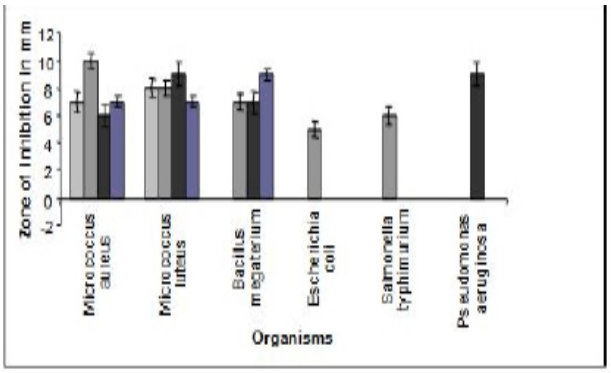

Figure 1. Antimicrobial activity of extracts using 30 µl volume of extracts using water ( ), Ethanol ( ), Methanol ( ), Acetone ( )

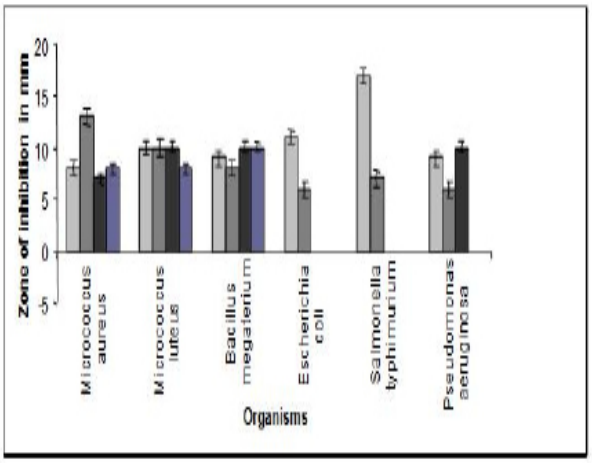

Figure 2. Antimicrobial activity of extracts using 50 µl volume of extract using water ( ), Ethanol ( ), Methanol ( ), Acetone ( )

In Figure 3 also all the extracts of 100 µl volume shows different antibacterial activities. Pseudomonas aeruginosa shows 13 mm zone of inhibition in water extract,11 mm in ethanolic extract and 16 mm zone of inhibition in methanolic extract whereas *Escherichia coli* is not inhibited by methanolic extract.

The presence of furfural in the extracts is confirmed by GCMS analysis (Figure 4) and the presence of caffeine is confirmed by similar analysis (Figure 5). Kokum showed antibacterial activity against gram positive and gram negative organisms. Antimicrobial activity of all kokum extracts against gram positive organisms increases as the volume of extracts increases. Water and ethanolic extracts showed significant zone of inhibition against gram negative organisms. *Pseudomonas aeruginosa* showed same result in methanolic extract whereas *Escherichia coli* and *Salmonella typhimurium* were not inhibited in methanolic extract. Acetone extract of kokum fails to inhibit the gram negative organisms.

Antimicrobial activity is primarily due to

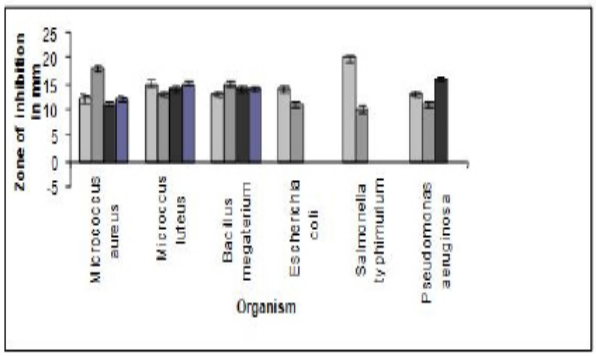

Figure 3. Antimicrobial activity of extracts using 100 µl volume of extract using water (), Ethanol (), Methanol (), Acetone ()

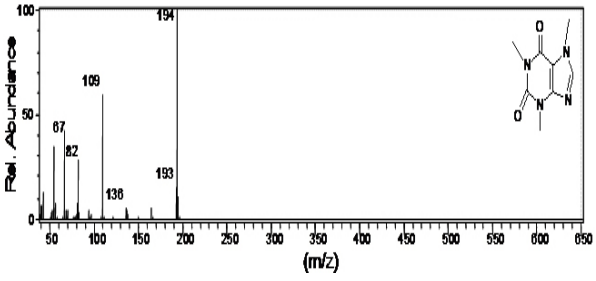

Figure 4. GCMS showing the presence of furfural in methanolic extract of kokum

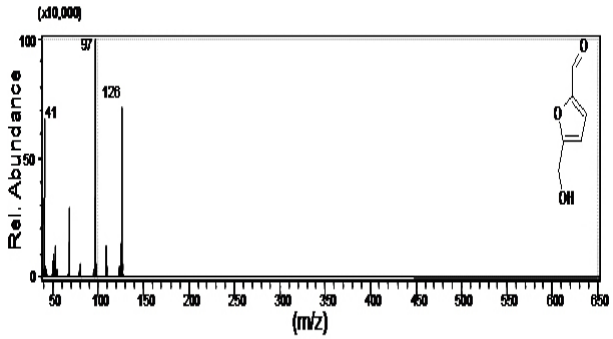

Figure 5. GCMS results showing the presence of caffeine in methanolic extract of kokum

presence of furfural in kokum extract. In many cases the main cause of indigestion has been attributed to imbalance in the normal microbial flora of the alimentary tract, like increased population of one type or intrusion of a harmful microbe. Restabilization of the flora resets the digestion and thus removes the problem associated with indigestion. If the problem is not a severe one then kokum juice can help to bring about the restabilization of the flora and thus rectify the problem of indigestion. These finding are concurrent with the similar results obtained earlier: ethanologenic *E. coli* (Miller *et al*., 2009), *Saccharomyces cerevisiae* (Taherzadeh *et al.*, 1997; Palmqvist and Hahn-Hagerdal, 2000). However, the quantity of the juice to be consumed should be just sufficient (depending on the individual). If consumed in excess, it can be harmful like furfural can inhibit some of the liver function like inhibiting alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase in the liver (Tobiaset *et al*., 2002).

The anthocyanins present (mass spectrographic results not shown) are also significant as antimicrobial agents. The prominent anthocyanin present is cyanidin -3- glucose. This is actually known for its anti inflammatory actions, but is significantly antibacterial in its characteristics against gram positive bacteria. That is why the extracts of kokum are often used to cure dermatitis or other mild skin infections, when applied topically. Similar anthocyanin has been reported from berry fruits (Cavanagh *et al.*, 2003), *Punica granatum* (Naz *et al.*, 2007).

Kokum extract acts as a nerve reliever due to the presence of caffeine. Again taken in small amount caffeine will act as a selective antagonist for adenosine 2A thus stimulating locomotor nerves. If Kokum is taken in excess then high dose of caffeine will inhibit locomotor nerves (Hsu *et al*., 2010) and the person may fall asleep.

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