British Journal of Pharmacology and Toxicology 1(1): 29-32, 2010
ISSN: 2044-2467

Submitted Date: May 16, 2010 Accepted Date: May 27, 2010 Published Date: June 20, 2010

Characterization of Some Antimicrobial Substances from Seed Coat of *Tamarindus indica* Linn.

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Abstract: *Tamarindus indica* is a well known fruit used widely in India, as it forms a seasoning in many foods. However, its preservative nature is very short lived as both malate and tartarate can be easily metabolized by many microorganisms. In this study an attempt has been made to investigate the inhibitory effect of the seed coat extract prepared in methanol, ethanol and acetone which showed the presence of other substances like furfural and its derivatives, levuglucosan, tetrazene, cyclohexasiloxane, dioxalene, etc. The microorganisms used were *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*.

Key words: Antimicrobial activity, cyclohexasiloxane, dioxalene, levuglucosan, *T. indica*, tetrazene

INTRODUCTION

Abukakar *et al*. (2008) had studied the phytochemical analysis and antibacterial activity of aqueous pulp extract of *Tamarindus indica*. phytochemical constituents present in extract were found to include saponins (2.2%), alkaloids (4.32%) and glycosides (1.59%). Saponin has detergent properties and serves as lytic agent. These are well known to exhibit anti-inflammatory properties, while alkaloids and glycosides aid in defense mechanism of the plant i.e. act as phytoprotective agent against invading microorganism. Aqueous extract of *Tamarindus indica* has been reported to show the presence of alkaloids, which are formed as metabolic by product, having antibacterial activity against all the tested bacteria in the order of sensitivity *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* with the exception of *Salmonella typhimurium* (Doughari, 2004). Isu (2005), reported that, the acidic nature and the antibacterial activity of *Tamarindus indica* suggest its usefulness in preservation of food items which get contaminated by *E. coli* and other Gram negative rods as well as is the management of cases of food infection by this pathogen.

Damien and Muhammad (2008) investigated the crude aqueous and ethanol extract of *Tamarindus indica* for antibacterial activity. The susceptibility of 5 clinical bacterial isolates against these two crude extracts was determined using the disk diffusion method. The ethanol extract produce strong antibacterial activity against *E. coli*, *Klebsiella pneumonia*, *Salmonella parathyphi*, *Pseudomonas aeruginosa. Staphylococcus aureus* was resistant to the extract. The aqueous extract has the least antibacterial activity compared to ethanol extract except against *Pseudomonas aeruginosa*. The antibacterial activity of the extract against the test bacteria suggest that there is a scientific basis for utilization in traditional medicine for the treatment of some bacterial infections as claimed by traditional medical practitioners. Medicinal uses of *Tamarindus indica* are numerous. The pulp is used as antipyretic, laxatives and carminatives, alone or in combinations with limejuice, honey, milk, dates, spices and camphor (Jayaweera, 1981).

The average pH of the pulp was 1.27 indicating the acidic nature of extract. Traditionally, the uses of plant preparation as chemotherapeutic agents were based on the experience (Sofawara, 1993). Martinello *et al*. (2006) reported that, dietary modifications with the pulp of *Tamarindus indica* may significantly reduce Cardiovascular Disease (CVD) risk factors, including cholesterol and atherosclerosis. Topically it is used as an antiscrobutic, when mixed with salt to treat rheumatism. In Southeast Asia it is administered to alleviate sunstroke, dasine poisoning and alcoholic intoxication (Morton, 1987). He further reported that, pulp is often used to allay thirst as it is nutritive and forms useful drinks given to person recuperating from sickness to keep their bowels regular. The acids present in the pulp include tartaric acid, citric acid and malic acid. The other constituents are potassium bitartarate, pectin, gum, water, and parenchymatous fiber.

Antimicrobial effect of polyphenolic compounded extracted from *Tamarindus indica* L. has been reported by Aengwanich *et al*. (2009). Lewis and Elvin-Lewis (1977) reported that, the bacteriostatic effect of extract on the growth of microorganism could be attributed to the presence of some phytochemicals that were found present in the plant extract. The fruit pulp is used as seasoning as a food component and in juices.

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In this investigation an attempt has been made to see the antimicrobial activity of the seed coat extract against certain bacterial species most commonly encountered in food poisoning cases among certain communities which live in remote parts of many developing nations and has little or no access to modern medicine to control the out beaks of food poisoning.

MATERIALS AND METHODS

Preparation of extract: The experiments were conducted between the period June 2009 to March 2010, in our laboratory. The fruits were obtained from all over the country.

The crushed seed coat was taken in mortar and pestle and different solvents like acetone, methanol and absolute ethanol, were added individually. These were then crushed well in the respective solvents. These were then filtered and the filtrates were centrifuged at 3622xg for 10 min and supernatant were used for further study.

Microbial cultures used to check antimicrobial activity: The microorganisms used were as follows, *Staphylococcus aureus* NCIM -5021, *Pseudomonas aeruginosa* NCIM-2036 and *Salmonella typhimurium* NCIM-2501.

Medium used: Nutrient agar medium and a mineral based medium were used in all further studies. The media compositions are as shown in Table 1 and 2.

Antimicrobial testing: Sterile molten nutrient agar at around 40°C, was taken into which different cultures was added and poured in sterile Petri plate and allowed to be solidified. After solidification 4 mm wells were prepared. In these wells solvent extracts of the seed coat were added. The plate was incubated overnight at 37°C. After incubation the zones of inhibition were measured and recorded. Respective solvent controls were also run simultaneously.

The above procedure was repeated using mineral based medium with added yeast extract at 0.02%.

Effect of different pH was observed on the antimicrobial effect using nutrient agar and mineral based medium. The different pH values were 5.0 and 8.0.

Detection of phytochemical: The different phytochemicals present in the solvent extracts of seed coat of tamarind were detected by GCMS.

Like wise the substances present in cell free mineral based liquid medium was extracted with methanol was analysed by GCMS and compared with a sterile medium containing tamarind pulp.

Table 1: Composition of nutrient agar medium

S. No.	Components	Percentage (%)
1	Peptone	1
2	Yeast extract	1
3	NaCl	0.5
4	Agar	2.5

Table 2: Composition of Mineral base medium

S. No.	Composition	Percentage (%)
1	Sodium nitrate	0.2
2	Dipotassium hydrogen phosphate	0.1
3	KCl	0.05
4	Glucose	1
5	Yeast extract	0.02
6	Agar	2.5

Table 3: Bioassay conducted with nutrient agar medium

Organisms	Methanol (mm)	Ethanol (mm)	Acetone (mm)
Pseudomonas aeruginosa NCIM-2036	11	16	7
Salmonella typhimurium NCIM-2501	9	7	7
Staphylococcus aureus NCIM-5021	8	15	6

Table 4: Bioassay conducted with mineral based medium

Organisms	Methanol (mm)	Ethanol (mm)	Acetone (mm)
Pseudomonas aeruginosa NCIM-2036	18	9	7
Salmonella typhimurium NCIM-2501	15	14	10
Staphylococcus aureus NCIM-5021	16	14	11

Table 5: Bioassay conducted with nutrient agar medium at pH-5

Organisms	Methanol (mm)	Ethanol (mm)	Acetone (mm)
Pseudomonas aeruginosa NCIM-2036	50	45	50
Salmonella typhimurium NCIM-2501	52	40	50
Staphylococcus aureus NCIM-5021	45	35	40

Table 6: Bioassay conducted with mineral based medium at pH-5

Organisms	Methanol (mm)	Ethanol (mm)	Acetone (mm)
Pseudomonas aeruginosa NCIM-2036	19	13	13
Salmonella typhimurium NCIM-2501	17	15	16
Staphylococcus aureus NCIM-5021	15	14	19

Table 7: Bioassay conducted with nutrient agar medium at pH-8

Organisms	Methanol (mm)	Ethanol (mm)	Acetone (mm)
Pseudomonas aeruginosa NCIM-2036	27	9	17
Salmonella typhimurium NCIM-2501	24	13	15
Staphylococcus aureus NCIM-5021	10	10	14

Table 8: Bioassay conducted with mineral based medium at pH-8

Organisms	Methanol (mm)	Ethanol (mm)	Acetone (mm)
Pseudomonas aeruginosa NCIM-2036	13	8	32
Salmonella typhimurium NCIM-2501	15	12	31
Staphylococcus aureus NCIM-5021	22	13	30

RESULTS

It can be seen from Table 3, that *S. aureus* and *S. typhimurium* showed maximum sensitivity to methanolic extract, *P. aeruginosa* was very sensitive to

Table 9: GCMS analysis of the extracts of the seed coat after and before inoculation

		After incubation with at 37°C for 144 h		
Methanol extract seed coat	Medium control	*Staphylococus aureus* NCIM-5021	*Pseudomonas aeruginosa NCIM-2036*	*Salmonella typhimurium NCIM- 2501*
Tetrazene	4H- Pyran- 4- one, 2,3 - dihydro-3,5- dihydroxy- 6-methyl	1,3- Dioxolane, 2, 4, 5- trimethyl	4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl	Tetrazene
(levoglucosan)	2-Furancarboxyaldehyde, 5-(hydroxymethyl)	2furancarboxyaldehyde, 5(hydroxymethyl)	1,6- Anhydro- beta- D-glucopyranose (levoglucosan)	2Furancarboxalhyde, 5-(hydroxymethyl
2- Furancarboxaldehyde, 5-(hydroxymethyl)	4-methylmannose	4- methylmannose	Furfural (levoglucosan)	4- methylmannose (levoglucosan) 1,6Anhydrobeta- Dglucofuranose

ethanolic extract. Acetone extract was not that effective. This was when the medium of growth was nutrient agar.

However, when the medium was changed to mineral based medium all the 3 organisms showed maximum sensitivity to methanolic extract as shown in Table 4.

It is evident that when there is an additional stress like a lowered pH then on an average the methanolic extract showed maximum bacteriostatic effect, with nutrient agar as the medium (Table 5).

Again from Table 6, one can get to see that even in mineral based medium stress makes the methanolic extract very effective as a bacteriostatic agent.

The methanolic extract works well except for *S. aureus* as shown in Table 7 but when one examines the same in mineral based medium, then one gets to seen that acetone extract works well at pH 8 (Table 8).

The results in Table 9 shows the components that are present in the extract and after incubation with the respective organisms, it can be seen that most of these are not degraded by these organisms.

DISCUSSION

It can be seen that the seed coat extracts prepared in methanol, ethanol and acetone, inhibited the growth of *Staphylococcus aureus* NCIM-5021, *Salmonella typhimurium* NCIM- 2501 and *Pseudomonas aeruginosa* NCIM-2036. The phyto chemicals though not fit to be used as food preservative but may be developed to products like disinfectants. The methanolic extract contained Tetrazene, Furfural and Levuglucosan, of which tetrazene and furfural are known for their inhibitory effect on different bacteria. However, the inhibitory effect of methanolic extract was very prominent in case of all the 3 organisms. It can be seen that none of these organisms could degrade any of these substances. The presences of these substances were confirmed by GCMS findings. Besides these certain other compounds were also detected in the seed coat extract which were furanmethanol, cyclohaxasiloxane, dioxolane, butanediol, D-allose etc. These compounds were reported to be very inhibitory to microbial growth. The inhibitory effect was enhanced in acidic pH as seen from the results (maximum inhibition is seen at pH 5 and not so in pH 7 and 8). It was also noted that when yeast extract was added at 0.02% the stress was prominent at pH 8 and hence maximum inhibition was observed in this pH value.

CONCLUSION

It can be thus concluded that the seed coat extracts prepared in different solvents contain many bacteriocidal agents and if these could be properly blended with certain other phytochemicals, possibly one can get a good disinfectant. However, the most important factor that might come in the way are the glycosides and sugars. From the results one can conclude that one of the physical factors that might help to increase the bacteriocidal effect would be the stress factor like pH.

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