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CHARACTERIZATION OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF THE LEAVES OF *SARACCA INDICA* AGAINST *E.COLI* NCIM 2832 AND *M. AUREUS* NCIM 5021.

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ABSTRACT

Saracca indica is a terrestrial tree found in tropical rain fed areas across the world. In India it is mostly found in peninsular region and in the foot hills of Himalaya mountain range. Till date its bark has found tremendous pharmacological application due to the presence of sterols like ergosterol which is a precursor of certain prostaglandins. Further investigations so far have indicated good antimicrobial activity too but reports of the application of this are still scanty. The objective of this investigation was not only to see whether the leaf extract also had antimicrobial activity, but to characterize such properties. The work was carried out by noting the difference in growth patterns of two most common opportunistic pathogens like *Escherichia coli* and *Micrococcus aureus*. It was observed that the growth rate of these two organisms was reduced using the ethanolic extract. This was primarily due to the presence of myristic acid and palmitic acid in the ethanolic extract, as revealed in the GCMS study. There was no effect on the growth pattern of *E. coli* by the aqueous extract. However, surprisingly, aqueous extract served as a growth stimulator for *M. aureus.* This was because both these fatty acids were absent in the aqueous extract and there was good amount of readily assimilable reducing sugars present in the aqueous extract.

KEY WORDS: Ashoka, antimicrobials, prostaglandins, E.coli, M.aureus.

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INTRODUCTION

Saraca indica is a commonly occurring plant mostly found in the peninsular India. It also occur in other parts of the world too. It has wide spread medicinal use, like its effect on the endometrium of the uterine wall due to the presence of ergosterol present mostly in the bark of the tree [1,2]. There are plenty of reports of its medicinal uses obtained from the bark. However, there is little report about the pharmacological properties of the leaf extracts. Beside ergosterol, there are reports of certain fatty acids also found in the bark. These are mainly palmitic acid, nonanoic acid, and phenylethanol beside certain proanthocyanidins. There are evidences of presence of Myristic acid in the bark extract of this plant to the concentration of nearly 19%[3]. This is a potent antimicrobial substance. Another essential fatty acid found in the bark extract of *Saraca indica* is arachidonic acid (an omega 6 fatty acid) which is normally derived from linoleic acid [4]. This is necessary for several body functions like signaling inflammation as well as an anti inflammatory substance. Beside this arachadonic acid has been shown to have anti bacterial properties against *Streptococcus mutans* [5,7.].Another anti microbial compound that has been reported from the bark of this tree, is lactam nonanic acid [6].

MATERIALS AND METHODS

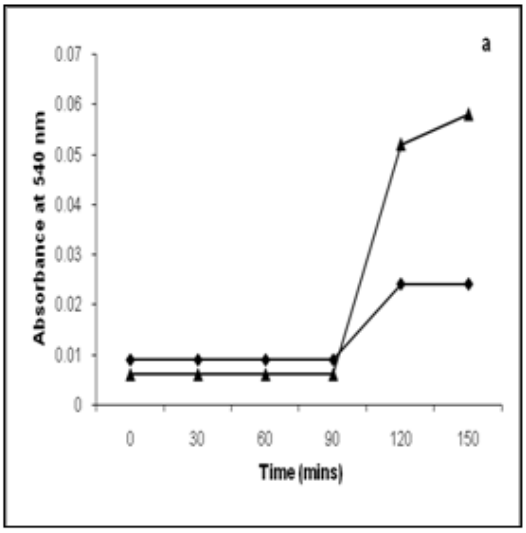
The fresh leaves of *Saraca indica*, visibly free from disease, were collected locally**.** The leaves were washed thoroughly 2-3 times with potable water and then air dried. Dried leaves were crushed in a blender. These were then soaked (25 g) separately in 200ml of ethanol and also in similar quantity of distilled water for 24hrs. The slurry was coarse filtered to remove the extracted powder. The filtrate was centrifuged at 7000 x g for 10mins. The clear supernatant was carefully decanted and stored at 4°C for further studies. The bacterial cultures used to study the antimicrobial activity of the extracts, were *Escherichia coli* NCIM 2832*, Micrococcus aureus* NCIM 5021. This was carried out by checking the growth pattern of the organisms in liquid medium containing meat extract 0.5%, peptone 1% and NaCl 0.5% at pH 7.0. The organisms were added at 10% level (v/v), with a cell density of 6 x 10^8 cells/ml. The absorbance was measured at 540 nm.

Statistical analysis

all the experiments were repeated 3 to 5 times till the data obtained were statistically valid as analyzed of the variance with Tukey Kramer multiple comparison test.

RESULTS

The objective of this study was not to just see whether the extracts were inhibiting the growth of the above mentioned organisms (by using routine techniques like agar well methods), but also to find out exactly what was the response of the organisms growing in presence of the extracts. Keeping this in mind, growth pattern studies were conducted as shown below.



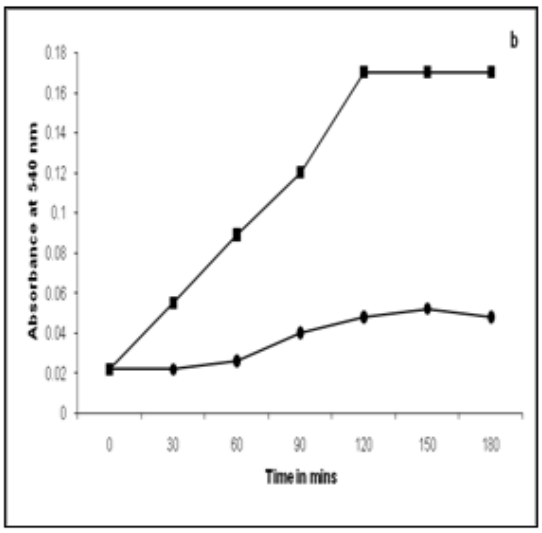
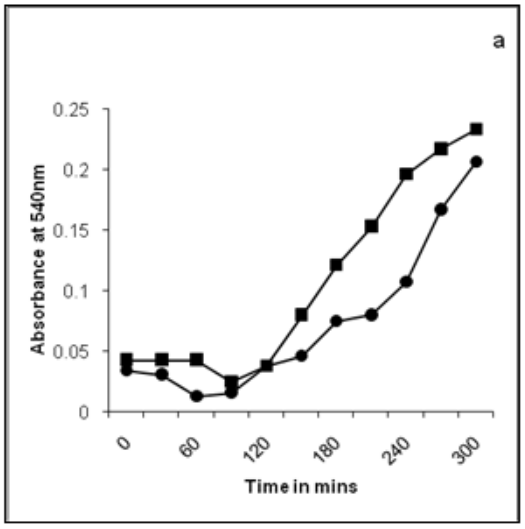


Figure 1 a: Growth pattern of *Micrococcus aureus* in absence of ethanolic extract of the leaves of *Saraca indica* (▲) and in presence of the ethanolic extract (♦).

Figure 1 b: Growth pattern of *Escherichia coli* in absence of ethanolic extract of the leaves of *Saraca indica* (■) and in presence of the ethanolic extract (●).

It can be seen from the Figure 1a and 1b that actually both the organisms did not get inhibited but had a reduced growth rate. On the other hand when the growth patterns of the 2 organisms were examined in presence of aqueous extract of the leaves, where it was observed that there was stimulation of growth of the organisms. This is as shown in Figure 2a and 2b.



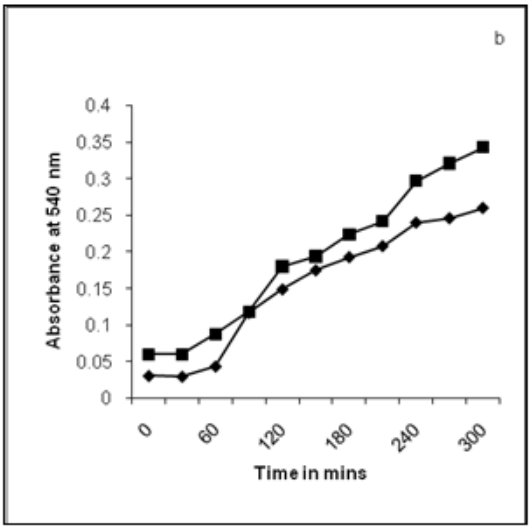


Figure 2a : Growth pattern of *Escherichia coli* in presence of aqueous extract of the leaves of *Saraca indica* (■) and in absence of the aqueous extract (◙)

Figure 2b: Growth pattern of *Micrococcus aureus* in presence of aqueous extract of the leaves of *Saraca indica* (■)and in absence of the aqueous extract (♦)

The GC MS analysis showed the presence of Myristic acid, Palmitic acid and Phytol in the ethanolic extract (results not shown). The reducing sugar content of the aqueous extract

was 533.34 µg/ml, as compared to that of the ethanolic extract which was containing 237µg/ml.

DISCUSSION

Since the compounds were identified using GCMS only, fatty acids like myristic acid, palmitic acid and phytol were detected in the ethanolic extract of the leave. The reduced growth rate of *E. coli* and *M. aureus* was primarily due to the presence of myristic acid and to some extent by palmitic acid. These observations concur with the ones reported by Neogi et al[8]. Phytol being a diterpene also has antibacterial properties [9] and possibly this too had the effect of bringing down the growth rate. However, none of these compounds are soluble in water and hence, the absence of antimicrobial activity of the aqueous extract probably be due to this reason. The reason for taking to study the difference in growth pattern was due to the non-observance of inhibitory zone on agar plates when the inhibition studies were carried out using agar well method. Another important observation was the growth stimulatory effect on *M. aureus*. On examining the aqueous extract it was seen that there was significant quantity of reducing carbohydrates present which was unlike in ethanolic extract. This effect was not very prominent in case of *E.coli*. This meant that the carbohydrate was selectively used by *M. aureus* only.

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