ANTIMICROBIAL ACTIVITY AND MINIMUM INHIBITION CONCENTRATION (MIC) OF ACACIA NILOTICA EXTRACTED BY USING ACETONE, CHLOROFORM, ETHANOL, AND METHANOL SOLVENTS

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ABSTRACT: This research studied the Acacia nilotica fruits (named Garad in Sudan) extract as antimicrobial agent. Samples were well prepared by garbling in powder form, extracted by solvent solution using acetone, chloroform, ethanol, and methanol, Acacia nilotica (fruit) extract was punched on the Erechie - coil and a Staphylococcus aureus organism which often found on the wound skin. The zone area of inhibition in both E. coli and S. aureus, was best found to be determined, methanolic extract showing the highest activity and more significant to inhibit the organisms compared to the other solvents herbal extract.The minimum inhibition concentration(MIC) is also determined at varying concentrations and it is clear that MIC result is good and fall between 40 &100mg/ml for E. coli , 50 & 100 mg/ml for S. aurous . GC – MS analysis was done for the herbal extracts in order to confirm the presence of the bioactive components.

المستخلص- هذا البحث اختص بدراسة عصارة ثمار شجرة السنط (القرض في السودان) كمضاد للجراثيم. وقد تم إعداد العينات جيدا بالطحن والغربلة لثمرة القرض بعد نزع بذورها وتحويلها الي شكل مسحوق و استخلاص المادة الفعالة منه باستخدام مذيبات الأستون ,الكلوروفورم, الايثانول والميثانول وتم حقن المستخلص في وسط ميكروبي لميكروبي القولونية والكائنات المكورات العنقودية الذهبية التي غالبا ما توجد في الجروح الجلدية لتحديد المستخلص الاكثر نشاطا لقتل الميكروب وذلك بواسطة قياس المساحة المتأثرة حيث وجد ان مستخلص الميثانول هو الأكثر نشاطا وفاعلية لقتل الميكروب , تم تحديد اقل تركيز لقتل الميكرويات وذلك باستخدام تراكيز مختلفة بواسطة طريقة الانتشار بالأغار حيث وجد ان أكثر فاعلية لأقل تركيز تنحصر بين 40% الي 100% لميكروب القولونية و50% الي 100% للعنقودية وتم التعرف علي المركبات الكيميائية النشطة في مستخلص ثمرة القرض باستخدام جهاز الفصل الكروماتوقرفي الغازى.

Keywords: *Acacia nilotica , methanol E. coli , S. aureus, herbal*

INTRODUCTION

Herbal coating techniques are nowadays spread all over the world, herbs are naturally available, they can be extracted without using any sort of chemicals and coated to textiles as medicinal material treatment for skin diseases among people[1]. These herbs are applied directly to the fabric as natural ingredients. Herbs do not pollute the environment through contamination of water resources. Herbs can also give all kinds of dye shades red, yellow, brown, orange and green and so forth [2] .Herbal textiles are eco-friendly and can they give away certain residues that can further be used for making other environmentally friendly products. The solids as well as liquid waste from herbal dyeing can be recycled to be used as manure in the field. Most of these herbs used dyeing of herbal textiles are cultivated in South East Asian countries such as India, Pakistan, and Bangladesh as well as in Africa[3-4]. A vast amount of water and chemicals is consumed in the process of dyeing and printing of textiles, numerous volatile agents are released into the atmosphere that is particularly harmful to our health. From an environmental point of view, the clothes we

wear and the textiles they are made from can cause a great deal of damage [5]. Most of the chemicals involved in the present manufacturing technology have been poorly tested for their toxicity, which is a complex biological phenomenon [6]. Many attempts have been made to develop medicinal herb extract treated garments using alternate medicinal concepts to cure selected disease. Many herbs have the potential of curing diseases like allergic dermatitis, psoriasis, asthma, liver disorders, headache, and joint pains [7]. There are many natural dyes obtained from plants that exhibit strong antimicrobial properties. Therefore, coating of antimicrobial plant natural dyes and bioactive plant extract on to cotton fabrics is an emerging technology in the production of medical cloths [8]. Textiles with anti-microbial finish not just protect the fabric, but also the user from microbial infestation. Hygiene has become the priority on textiles as they are termed the second to skin and are closer to the human body. This aspect calls for greater importance given to anti-microbial in textiles [9]. In a research conducted to test the microbes it was found that bacteria isolated from clothing are similar to those isolated from normal skin [10]. Herbalists tend to use extracts from parts of plants, such as roots or leaves but not isolate particular phytochemical. They argue that the different phytochemical present in many herbs will interact to enhance the therapeutic effects of the herb and dilute toxicity [16]. Many of the anti-microbial agents available in the market are synthetic based and may not be environmentally friendly. Due to this, many of the consumers are opting for herbal anti-microbial finishes for textiles [11]. It must be ensured that these substances are not only permanently effective, but also that they are compatible with the skin and environment.

Herbals extracts can improve to greater extend the following properties of textiles i.e. water repellent, wrinkle resistance, anti-bacteria, improvement of dye ability. Soil resistance, anti-static, UV-protection, flame retardation. All studies stated that herbals have strong, efficient and more safety when used as antimicrobial compared to synthetic drugs which often have side effects in the human body and in the environment. More than one study approved that Acacia nilotica products (leaves, roots, pods, outer layer of the stem) are better and more effective when used as an antimicrobial in drugs [13, 14, 15].

De and Ifeoma (De, N [17] has studied the phytochemical constituent and some antioxidant indices of ethanolic leaf extract of Azadirachta indica were evaluated, It shows that the plant has antioxidant activity, the phytochemical constituent explain why the plant has been used as antimicrobial agents for preventing and treating microorganism infections. Banso, A [18] showed the antibacterial activity of the extracts of Acacia nilotica assay against Streptococcus viridians, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Shigellasonnei using the agar diffusion method. This study shows antibacterial activity against all the above said organisms, but Bacillus subtilis most susceptible to the plant extract. Deshpande S.N [19] has been conducted study on ethanol and petroleum ether extract of the stem bark of Acacia nilotica; the results show both the extracts exhibited inhibitory action on the pathogens above mentioned. But; ethanol extract showed greater activity as compared to corresponding petroleum ether extract.

The main objectives of this study is to select suitable solvent for the extraction of antibacterial activity from Acacia Nilotica, to determine the minimum inhibition concentration of the extract and to characterize the extract chemically in order to determine and to assess the effective group or components from the extracts as a potential for antimicrobial textiles.

MATERIALS AND METHODS

Chemicals

The solvent methanol (Sigma – Aldrich, Mumbai), ethanol (Changshu Yangon, China), Acetone (Loba cheme, Mumbai) and Chloroform (Nice, Cochin) were used for extract preparation. Muller Hinton agar and agar purchased from Himedia (Mumbai) were used as the culture media.

Test Organisms

Bacteria

Clinical isolates (organisms) of Escherichia coli and Staphylococcus aureus obtained from the Department of Microbiology, Kovai Medical Centre & Hospital (KMCH), Coimbatore. All cultures were biochemically tested for purity and were used in this study.

Extraction

In aqueous extraction, 10g of dried powder was placed in 100 ml of distilled water and boiled for 6 h and then filtered by watchman filter paper No (1) [17]. The filtrate was condensed in boiling water bath and used for further analysis.

In organic solvent extraction, 10 g of dried powder was placed in 100 ml of organic solvent, namely petroleum ether (distillation range: 60–80 ◦C), methanol, ethanol, chloroform and acetone in separate conical flasks and kept in a rotary shaker at 150 RPM for 24 h [12]. Then it was filtered and the solvent was evaporated with rotary vacuum evaporator to make the final volume one-fourth of the original volume. It was stored at 4 ◦C for further analyses.

Ethanolic Extraction

20g of Acacia nilotica powder was weighed and mixed with 100ml of ethanol and kept in overnight. The filtrate was obtained by filtering these contents twice using Whatsman filter paper No 1. The clear filtrate was condensed using a rotary vacuum evaporator at 50°C for about 15 minutes.

The same process was repeated for other solvents, to prepare methanolic , chloroform and acetone extraction.

RESULT ANALYSES

GC – MS analysis was done for the herbal extracts in order to confirm the presence of the bioactive groups of the herbal extract. The analysis was performed in Thermo GC trace ultra version 5.0 at a flow rate of 1 ml/min. Helium was used as a carrier gas in a capillary non-polar column. Substances were identified by their retention times and mass spectra which were compared with library and confirmed with those reported in the literatures.

The chemical composition of methanolic acacia fruit Fig.1 extract was analyzed by GC-MS. In the GC-MS chromatography the major compounds present in Acacia were listed in Table (1). The retention time was calculated from 0-44.08 mins. The elution of a phytochemical (Pyrogallol, Dihydroxyphenol, Oxirane) was observed both in the extract the major constituents present were palmitic acid, Oxirane, 4-Nitrosoquinoline, Piperidin-2, 6-di-carboxylic acid, Silanol, Phenol, Sesquirosefuran, Celidoniol Dotriacontane, Pyrogallol. The presence of these phytochemicals indicates the presence of the active factors of the acacia. The flavanoids, alkaloids and the sesquiterpenoids present in the fruit extract was responsible for the antimicrobial activity of the fruit extract. The presence of these phytochemicals was in good agreement with the results of preliminary phytochemical analysis. See Fig.2 to Fig.9

Antimicrobial Susceptibility Testing

The agar well diffusion method was used for antimicrobial susceptibility testing . 0.1 ml of diluted inoculums (105 CFU/ml) of test organism was spread on MHA (Muller Hinton Agar) plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100 µl of plant (A.seyal) extract and solvent blanks. The plates were incubated for 18 h at 37 ◦C for bacteria and 72 h at 27 ◦C for fungal specimens. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. The differences in the zones of inhibition between different extracts of A. Nilotica were interpreted.

Determination of the Minimum Inhibitory Concentration (MIC)

The fruit extracts of A. Nilotica which showed significant antimicrobial activity in the antimicrobial susceptibility testing A stock solution of 100 mg/ml of the condensed extract was prepared. This was serially diluted to obtain various ranges of concentrations between 10 µg/ml and 100 µg/ml. 0.5 ml of each of the dilutions of different concentrations was transferred into a sterile test tube containing 2.0 ml of nutrient broth. To the test tubes, 0.5 ml of test organism previously adjusted to a concentration of 105 cells/ml was then introduced. A set of test tubes containing nutrient broth (2ml) alone were used as control. All the test tubes and control were then incubated at 37 ◦C for 18 h. After the period of incubation, O.D. (optical density) readings of the tubes were measured at 420 NM Spectrophotometer were selected for determination of MIC [17]. See Table.2 Spectrophotometer –Robonik model Readwell Flisa plate analyzer-Sr No (RT0830215RBC) Fig 10.

RESULT AND DISCUSSION

Antimicrobial Susceptibility Testing:

Evaluation

The MHA plates were inoculated by test organisms then filled by acacia nilotica extract (acetone, chloroform, ethanol, and methanol). In both E. coli and S. aureus, the zone area of inhibition was measured. Methanol extraction showed higher activity and more significant to inhibit the organisms compared to the other solvent herbal extract, therefore methanol extract will be selected as the suitable extract for test application in this study Fig (11,12) Table (2).

Determination of the Minimum Inhibitory Concentration (MIC)

Evaluation

Different dilutions of 100mg/ml of methanol extract transferred to 0.5ml of test organism tubes with a set of tubes containing broth as control, all sample test tubes and control was incubated and evaluated using a spectrophotometer. The minimum inhibitory concentration (MIC) of methanolic should be equal or less compared to the control MIC value. and it is clear that MIC result is good and fall between 40 &100mg/ml (E. coli) , and fall between 50 & 100 mg/ml for (S. aurous) Fig. (13).

CONCLUSION

The Acacia Nilotica plant was selected for this study, susceptibility testing was carried out by ethanol, methanol, acetone and chloroform as solvent and analyzed for the antimicrobial activity

- Susceptibility testing showed that the methanolic extraction is selected for its high activity and efficiency against E. Coli and S. aurues.
- Minimum concentration, inhibition is done by fraction at 100ml/well concentrations and it is clear that MIC result is good and fall between 40 &100mg/ml (E. coli) , 50 & 100 mg/ml for (S. aurues) .
- The Acacia nilotica methanolic extract is good potential for antimicrobial textiles.

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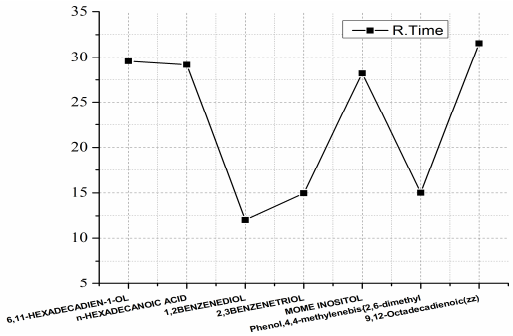
Table.1: The chromatogram of *Acacia nilotica* fruit-(Major ingredients)

Peak#	R. Time	I. Time	F. Time	Area	Area%	Height	Height %	A/H	NAME
1	29.554	29.283	30.808	414036510	30.57	9473746	15.54	43.70	6,11-HEXADECADIEN-1-OL
2	29.163	28.425	29.283	364717061	26.93	10456097	17.15	34.88	n-HEXADECANOIC ACID
3	12.067	11.833	12.383	24994906	1.85	1106997	1.82	22.58	1,2BENZENEDIOL
4	14.951	14.567	14.983	275327170	20.33	16448835	26.98	16.74	2,3BENZENETRIOL
5	28.222	27.900	28.375	61204835	4.52	4058320	6.66	15.08	MOME INOSITOL
6	15.010	14.983	15.717	159769591	11.79	14200377	23.30	11.25	Phenol, 4,4-methylenebis {2,6-dimethyl
7	31.565	31.358	31.833	22515363	1.66	2002639	3.29	11.24	9,12-Octadecadienoic (zz)

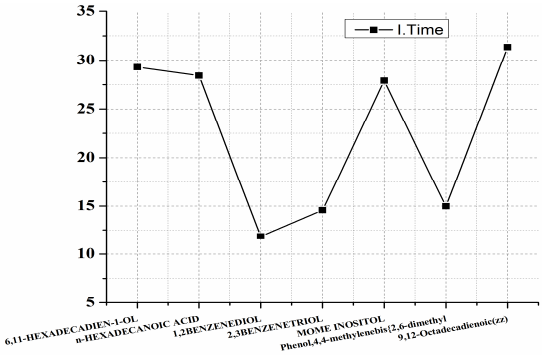
Table 2: Preliminary screening of herbal extract for antibacterial activity (Well diffusion test)

S.No	Test Organisms	Different solvent herbal extract (2g/10ml) Zone of inhibition (mm)				Chloramphenicol (Standard Antibiotic)
		Acetone	Chloroform	Ethanol	Methanol	
1	*E. coli*	8	6	6	10	12
2	*S. aureus*	11	11	11	12	13

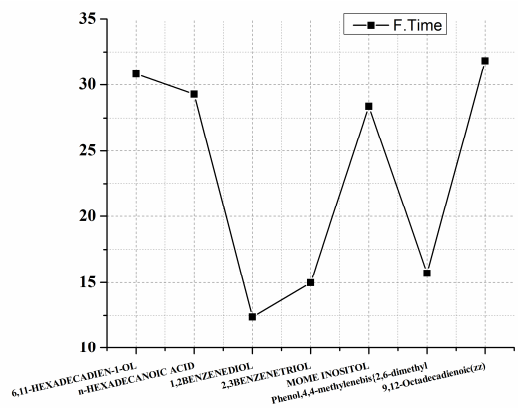
Figure 1: (a) Acacia nilotica pod (fruit +seeds) Fig1 (b) Fruit powder



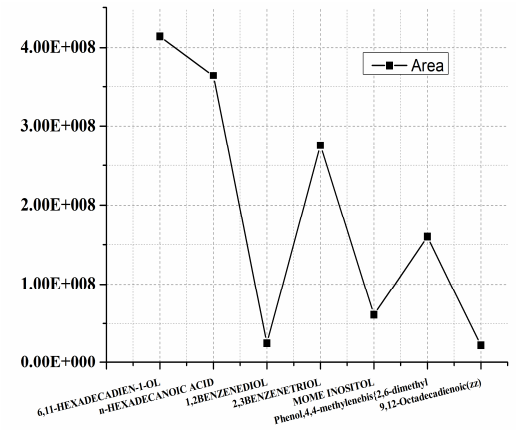
Fig(2)-Retention Time



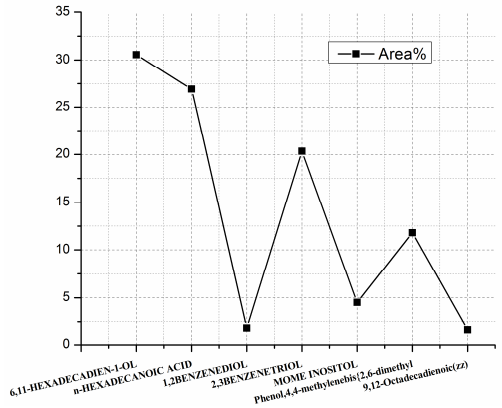
Fig(3)-Final Time



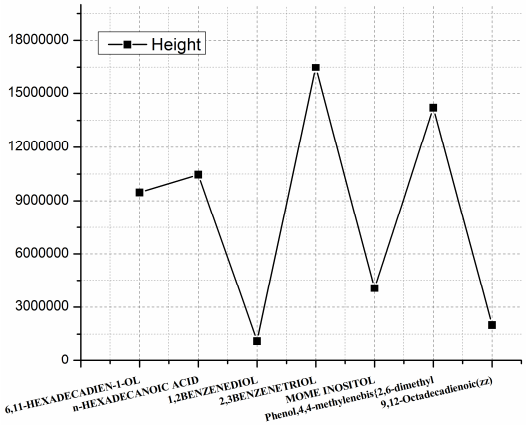
Fig(4)-Final Time



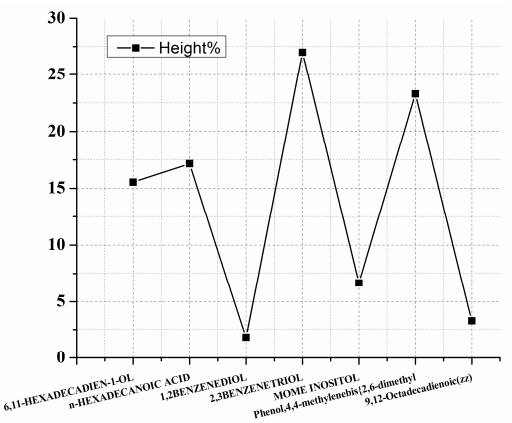
Fig(5) Area



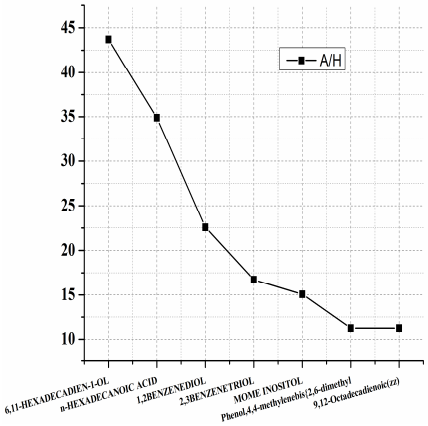
Fig(6)-Area %



Fig(7)-Height



Fig(8) Height



Fig(9)-Area/height

Figure 2 -9: Chromatogram of *Acacia nilotica* fruit-(Major ingredients)

Figure 10: Spectrophotometer –Robonik-model Readwell Flisa Plate Analyzer

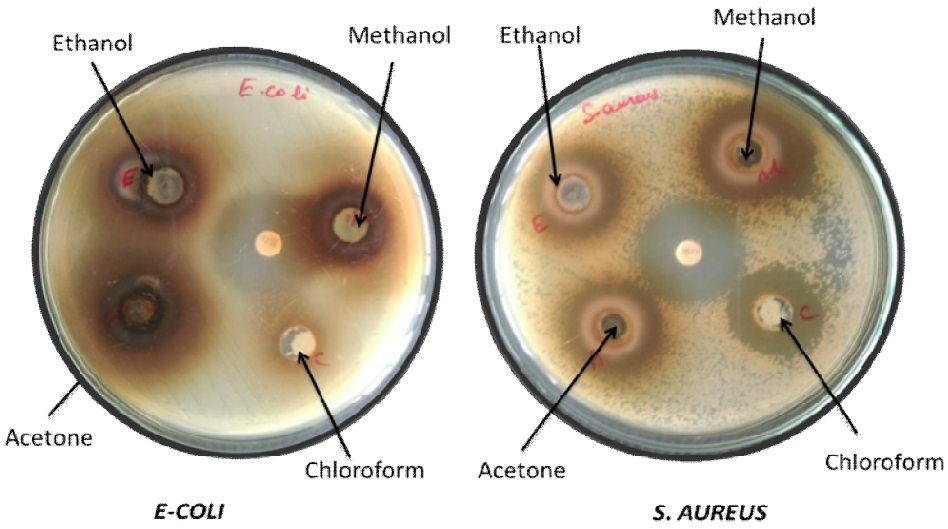


Figure 11: Preliminary screening of herbal extract for antibacterial activity (Well diffusion test)

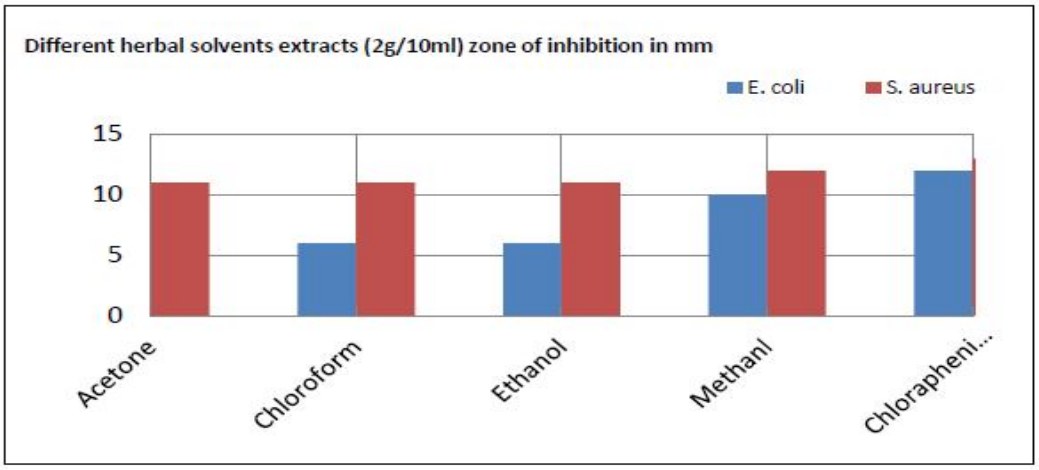


Figure 12: Different solvent extract(2g/10ml)zone of inhibition in mm

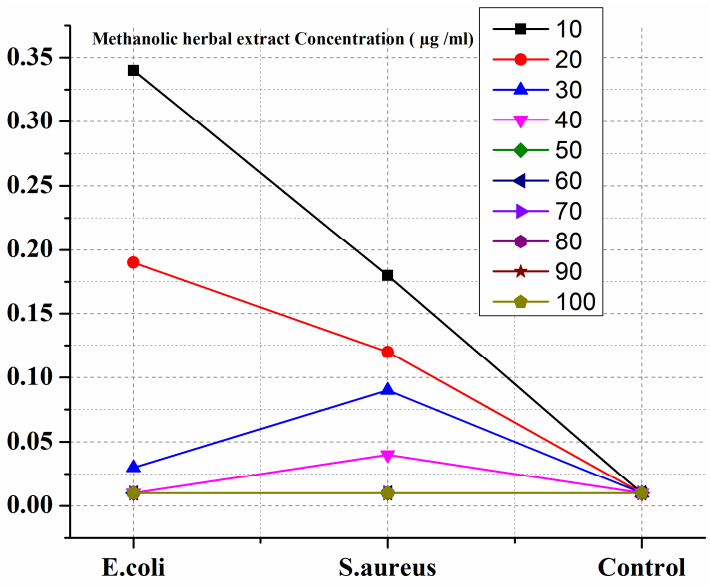


Figure 13: Minimum Inhibitory Concentration of the Herbal Extracts.