

ANTIBACTERIAL ACTIVITY OF HEALTHCARE PRODUCTS “LOWSONIA INERMIS AND INDIGOFERA TINCTORIA”

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ABSTRACT

Lowsonia inermis commonly known as” **HENNA**” belongs to the family Litheraceae. Henna is widely used as herbal medical all over the world since centuries as an antimicrobial agent and is sometimes also applied directly to the affected area for dandruff, eczema, scabies, wounds, infectious diseases and helminthiasis and as herbal hair and skin dye. **INDIGO** dye is Indigofera tinctoria distinctively blue in color and it’s basically an organic compound. It’s a synthetic dye. Soxhlet and cold percolate extraction methods were investigated for isolation and biological evaluation of bioactive compounds of henna and indigo. Obtained results showed that soxhlet extraction at higher temperatures, the yield and recovery decreased due to the degradation of the thermos reactant compounds at several temperature conditions. Antibacterial effects of water, methanol extracts of L.inermis leaves and Indigo dye against clinical bacterial cultures were investigated and compared with standard antibiotics of Ciprofloxacin and Ampicillin. The bacterial isolates were treated with L.inermis methanol extracts were more effective than the water extract using standard method of NCCL, 2000.

KEY WORDS: Lawsonia inermis, Indigofera tinctoria , Extraction , Antibacterial activity, Herbal Medicine

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INTRODUCTION

Nature is full of beautiful colours that attract human attention. Natural dyes have been used since ancient times for dyeing of body, food, walls of caves, textiles, leather and objects of daily uses (Kramell, A. et al) India has a rich biodiversity and it is not only one of the world's twelve mega diversity countries, but also one of the eighth major center of origin and diversification of domesticated taxa (Iqbal Sanjeeda and Ansari Taiyaba N.*2014) In the past, painters had used natural dyes extracted from plants, insects, molluscs and minerals for their paintings. The unique character of their works were the result of using different mixtures of dyes and mordents, as varnishes and lacquers responsible for cohesion of the pigments and protection of the layers destroyed by environmental effects. Natural dyes were also used in clothing's as well as in cosmetic industry (Henna, Catechu), pharmaceutical industry (Saffron, Rhubarb) and in food industry (Annatto, Curcumin and Cochineal) (Mohd Yusuf .et.al) The present study aimed at evaluating the natural antimicrobial activity of *Lawsonia inermis* (*L. inermis*) and *Indigofera tinctoria* (*I. tinctoria*) against a reference and pathogenic clinically isolated strains compared with some antibiotics. Many studies on *Lawsonia inermis* leaves extractions showed that it had antibacterial activity against Gram positive bacteria and against Gram negative bacteria.

Natural dyes are used to impart colour to an infinite variety of materials such as textiles, paper, wood etc. , but also widely used in cosmetic, food and pharmaceutical industry. They have very wide range of medicinal importance in pharmaceutical industry. (B.CHENGAI AH* et.al)

Synthetic dyes are manufactured from organic molecules playing more and more important roles in our modern life with applications both in industry (e.g., paint industry) and also in scientific laboratories (e.g., fluorescent tracers and photoredox catalysts). Synthetic dyes are sometimes referred to as 'coal tar dyes', since they are manufactured from substances which until recently were only obtained from coal tar (Ghodsi Mohammadi Ziarani et.al)

Henna (*Lawsonia inermis*, family Lythraceae) is a shrub cultivated in Asian countries India, Sri Lanka and North Africa and contains the active dye named lawsone (2-hydroxy-1,4-naphthoquinone). Henna dye is obtained from the dried leaves, which are powdered and mixed with oil or water and are used to prepare hair and body dyes.(Ik-Joon Kang,Mu-Hyoung Lee et ,al) Henna, this is widely used in the cosmetic industry as dyeing agent. It has medicinal importance along with dyeing property (B.CHENGAI AH*et. al) the pigment lawsone (2-hydroxy-1, 4-naphthoquinone; CI 75480; Natural Orange 6), which is present at a concentration of <2% in henna leaves and natural henna preparations (Almeida P J et.al) In Arab countries, henna is used for medicinal purposes, for the treatment of boils and folliculitis, by mixing it with vinegar and making a hot poultice. It may also be used to reduce pain, swelling, and high fevers, and to treat alopecia, burns, headaches, and gastrointestinal symptoms; in addition to this, henna has alleged antimycotic, tuberculostatic and also ultraviolet A-blocking properties (Anton C. de Groot 2013) In the industrial sector, the powdered form is used by Muslims and Orthodox Jews, because they refuse to use synthetic dyes to tinge silk, wool, and cotton fabrics . In India, surgeons use henna as a durable preoperative skin marker (Mehendale V G et.al). In the past 15 years, a new mode of henna applications called temporary black henna tattoo also sometimes called skin painting or pseudo-tattooing , has become

fashionable. Black henna sometimes also termed blue henna, is the combination of red henna and PPD. No natural black henna exists in real. Some of these 'henna' preparations do not even contain red henna at all (Almeida P J et.al) PPD is added to henna to accelerate dyeing and drying process to strengthen and darken the colour, to enhance the design pattern of the tattoo, and make the tattoo last longer. (Anton C. de Groot 2013)

Indigo Dye is one of the most oldest textile dyes and was originally prepared from the plant material. The most effective plant-based process of producing indigo dye involved by the plant *Indigofera tinctoria*, which is been distributed throughout the tropical and subtropical regions of the world and particularly in India. In fact, Greeks referred to this dye as "indikon" which means it is "from India." (Gilbert et al. 2004) Nowadays, indigo is also chemically synthesized at a large scale to satisfy the demand for dyeing the jeans. The current indigo production processes are mainly based on fossil feed stocks (Andrea N. Fabara et. al) Indigo dye is mostly used to dye denim fabric in recent era (Ramya et al., 2008) . Its high toxicity has also been reported recently (Barka et al., 2008) . Because it is recalcitrant to the activated sludge system of decolorization, Bacterial, fungal, and enzymatic decolorization of indigo (Barka et al., 2008; Ramya et al., 2008; Birhanli and Yesilada, 2010; Terres et al., 2014)

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EXPERIMENTAL METHODS

HENNA

SAMPLE COLLECTION AND DRYING:

Lawsonia inermis (Henna) leaves were collected about 1kg from private gardens at Visakhapatnam city. The leaves were separated from the stem. These leaves were left to dry under shade at room temperature for about a week or two weeks. These leaves should be completely dry so that there no moisture present in them.

EXTRACTION OF LOWSONE:

The dried leaves are manually smashed until they are finely powdered. This powdered sample is weighed around 50 gm. as shown in fig: 1, and soaked in 1ltr of distilled water or tap water for about 24 hrs. in a henna jar. This soaking results the chemical compounds to drain down into water from the dry leaf powder. This reaction was carried out in a dark reaction at room temperature called cold mastication .This is the important step were the main colouring compound lawsone was observed in soaked water. This soaked sample was filtered after 24 hrs.' This filtration takes more time than a day. The filtrate was separated by removing the solid debris in one beaker and liquid matter in conical flask. This leaf debris can be used for other research purposes. And the liquid is collected about 500 ml in a flask.

DRYING:

The soaked liquid was poured a petri plat and left for drying as shown in the fig: 2 . Another method of extraction is by soxhlet extractor also a chemical way of extracting the lawsone compound.

- **Water extraction:**

About 100gm of henna dry leaves were extracted by water extraction. 1ltr water was added to 100gm of henna powder. 24 hours soaking was done and filtered the solution. The solid sample was used for other research and liquid was collected and poured in petri plate. This was left for drying in a shade.

- **Methanol extraction:**

About 100 gm. of Henna dry leaves were extracted by means of methanol extraction. 600ml of methanol was added for Henna dry leaf powder. Distillation process was

carried out in soxhlet extractor. The extracted liquid is poured in petri plat and allowed to dry in shade till it is completely powdered.



Fig. 1 - Dry powdered sample



Fig . 2 – Drying the henna methanol extract

ANTIBACTERIAL ACTIVITY:

Anything that destroys bacteria or suppresses their growth of their ability to reproduce heat Chemicals such as chlorine and antibiotic drugs all have antibacterial properties many antibacterial products for cleaning and hand washing are sold today such protect do not reduce risk of symptoms of the viral infections diseases in the other ways healthy persons this does not preclude the potential contribution of antibacterial products to reducing symptoms of bacterial diseases in the home. Antimicrobial assessment performed in vitro using the cat description method Antibacterial activity of Nano carriers for lawsone compound was tested in vitro by measuring zone of inhibition against suitable microorganisms such as Escherichia coli, Sthinogomonas paucimobilis and klebsiella pneumonia. The microorganisms of interest were grown on a compatible sterilized nutrient agar medium. Test composites on square plastic grids or disks were transferred on the solidified agar medium and incubated for 24 hrs.’ at 37 degree Celsius. After the incubation period, the zones of inhibition were observed in four directions for each sample.

TLC FOR LAWSONE:

The extracted compound of lawsone was taken as sample for testing the presence of compound by thin layer chromatography (TLC) method. The sample was taken and solvent was prepared. The TLC paper was marked to add the sample, to know the initial length. The

sample was added drop wise and dipped in the TLC solvent. Observe the results and note the length of the compound movement in TLC paper. RF is calculated according to the observed value

INDIGO:

SAMPLE COLLECTION: Sample was collected from healthcare laboratories and conducted antimicrobial and antifungal activity.

ANTIBACTERIAL ACTIVITY:

The antibacterial activity for Indigo sample was carried out by preparing nutrient agar medium. The two samples of Indigo were tested. After nutrient agar medium is solidified the disk diffusion method was carried out by making the sample diluted in water and methanol. The bacterium used in identifying the bacterial activity is *Escherichia coli*, *klebsiella pneumonia*, *Sphingomonas paucimobilis* strains. The activity is tested after incubation for 24hrs at room temperature in laminar airflow. If the inhibition is present the media shows the disk formation and if not present then there is no antibacterial activity present for the tested sample such as Indigo.



Fig . 3 Indigo extract

RESULTS AND DISCUSSION:

Two different types of antimicrobial activity test were conducted on three different bacterial strains selected for this study for the first test the Agar dilution method was used to determine the minimal inhibitory concentration (MIC) of antimicrobial agents which is the lowest concentration of the antimicrobial agent that will inhibit the visible growth of microorganism in question the second test was the disc diffusion method which is measurement of the diameter of a ring that forms around the colony after treatment. No formation of disk means that there was no antimicrobial activity and differing exists the size of the Ring indicates the degree of antimicrobial activity Agar dilution method is considered to be the reference method for their other antimicrobial susceptibility test because of its accuracy the disc diffusion method . The extracted henna gave about 15gms of lawsone compound per 1kg of henna leaves. The results showed that methanol and water showed results of inhibition to the tested strains of bacteria.

The antibacterial activity of Henna lawsone compound and Indigo dye was as follows.

TLC FOR LAWSONE COMPOUND:

Different samples of lawsone extract was collected and tested for Thin layer chromatography. The different samples are Henna methanol, Henna chloroform, and henna water. Amongst these three henna methanol showed highest Rf values compared to chloroform and water samples as shown in the fig .4

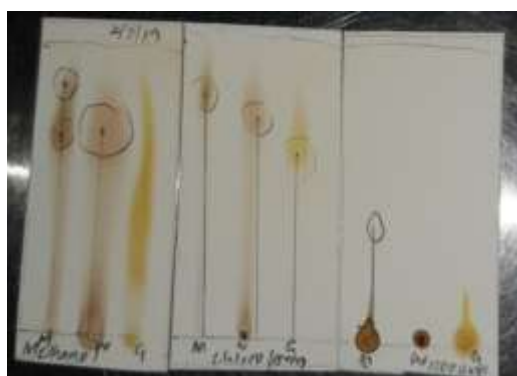


Fig . 4 Thin layer chromatography for extracted lawsone compound

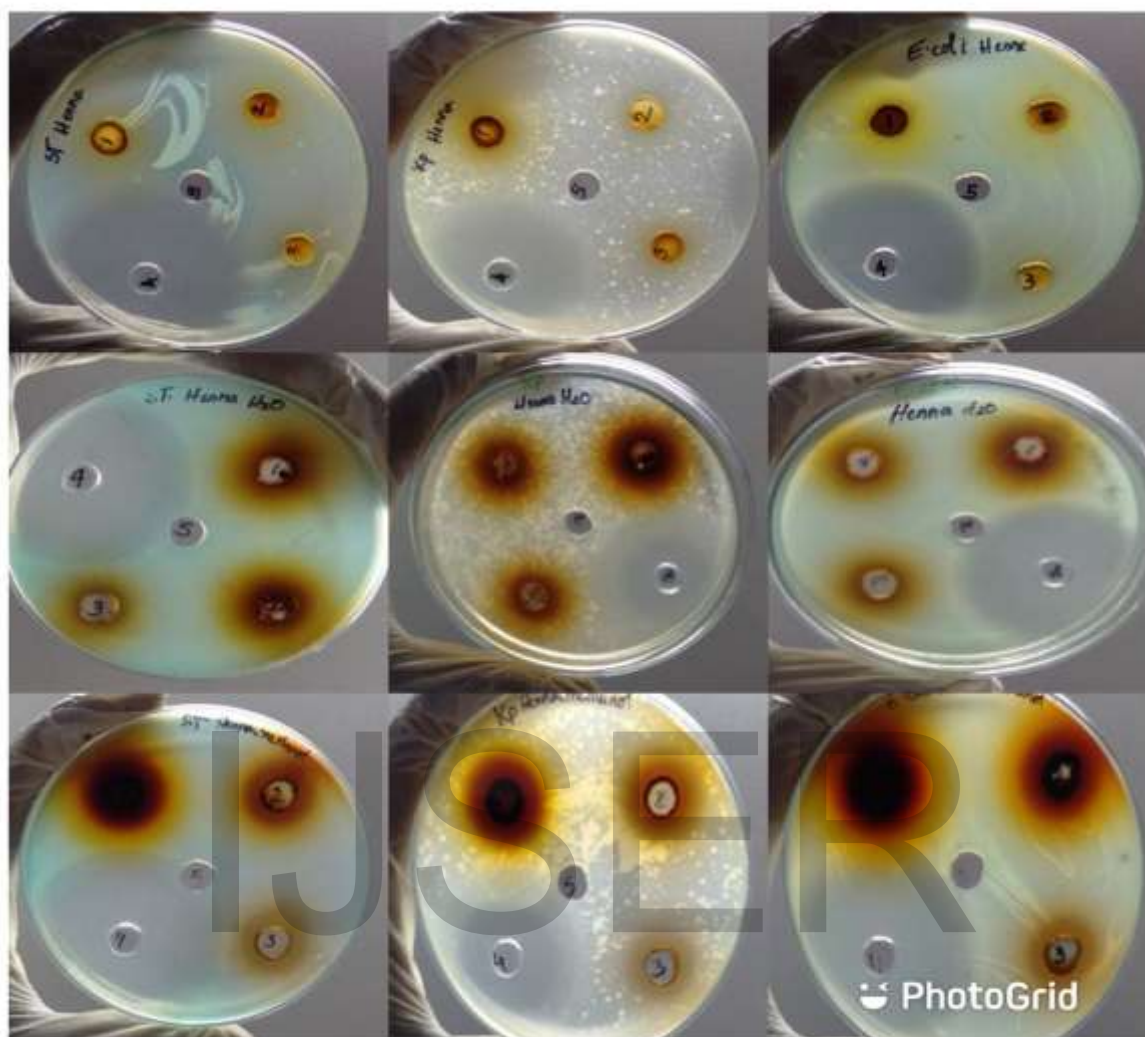


Fig. 5 Zone of inhibitions in Lawsonia inermis synthetic dye ,Henna water and Henna methanol extracts using bacterial strains such as Spingomonas paucimobilis, Klebsiella pneumonia , Escherichia coli

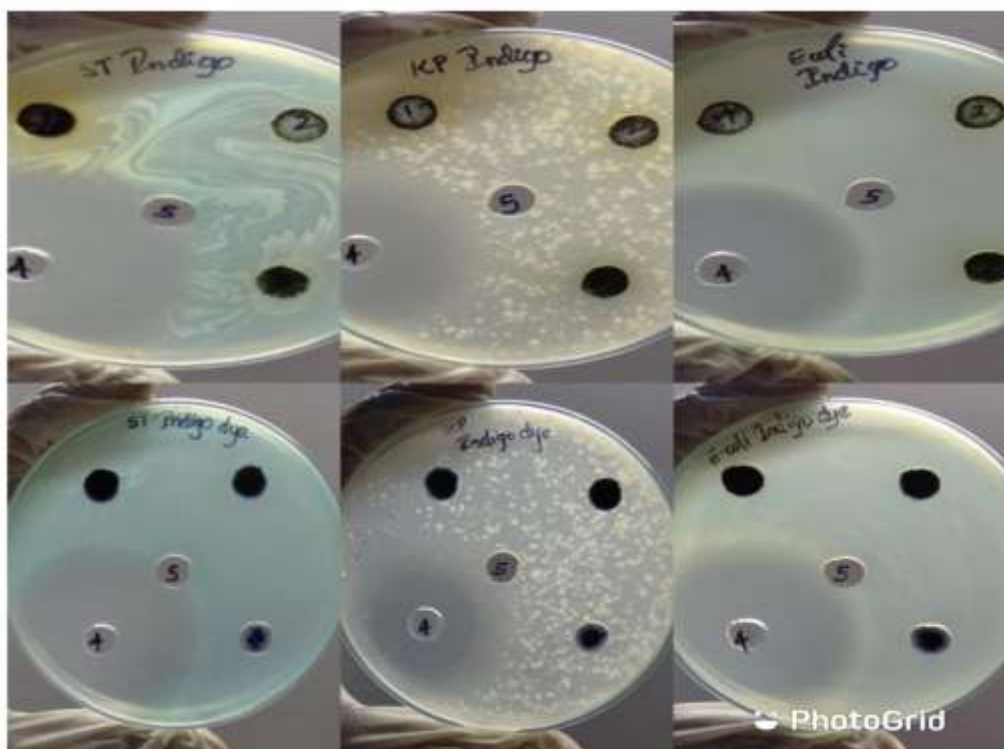


Fig. 6 Zone of inhibitions in Indigofera tinctoria synthetic dye extracts using bacterial strains such as Sphingomonas paucimobilis, Klebsiella pneumonia , Escherichia coli

Henna methanol , antibacterial activity by klebsiella pneumonia showed highest antibacterial activity with 13mm diameter.

Henna water, Antibacterial activity were klebsiella pneumonia showed highest antibacterial activity with 18mm diameter.

Indigo Antibacterial activity, 10mm is the highest activity seen in all the bacterial strains.

Tab : 1 Antibacterial activity zone of inhibition results table

Indigo	1000mg/ml	500mg/ml	250mg/ml	Antibody
Sphingomonas paucimobilis	10mm	9mm	8mm	40mm
Klebsiella pneumonia	10mm	9mm	8mm	40mm
Escherichia coli	10mm	9mm	8mm	40mm
Henna water	1000mg/ml	500mg/ml	250mg/ml	Antibody
Sphingomonas paucimobilis	11mm	13mm	12mm	40mm
Klebsiella pneumonia	18mm	15mm	14mm	37mm
Escherichia coli	14mm	13mm	11mm	40mm
Synthetic Henna	1000mg/ml	500mg/ml	250mg/ml	Antibody
Sphingomonas paucimobilis	12mm	11mm	10mm	40mm
Klebsiella pneumonia	12mm	13mm	12mm	40mm
Escherichia coli	11mm	10mm	9mm	40mm
Indigo dye	1000mg/ml	500mg/ml	250mg/ml	Antibody
Sphingomonas paucimobilis	11mm	11mm	10mm	40mm
Klebsiella pneumonia	11mm	10mm	07mm	40mm
Escherichia coli	11mm	10mm	09mm	40mm
Henna methanol	1000mg/ml	500mg/ml	250mg/ml	Antibody

Sphingomonas paucimobilis	12mm	11mm	10mm	40mm
Klebsiella pneumonia	13mm	11mm	10mm	40mm
Escherichia coli	13mm	11mm	10mm	40mm

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CONCLUSION:

The present work shows that plant extract such as Lawsonia and Indigo dyes were active against *E. coli*, *Klebsiella pneumoniae* and *Sphingomonas paucimobilis* that are pathogenic to humans where can be inhibited by Lawsonia and Indigo compounds. Indigo showed antibacterial activities in the tested strains. Some of the tested strain show significant differences in their responses to the extracts by disk diffusion method. Primary inhibition caused by the methanol extract water extract of Indigo was observed partially, therefore it can be hypothesized that the basic dye of Indigo, henna contain a wide spectrum of antibacterial compounds with maximum 18mm diameter in the media.

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