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EXTRACTION AND ANTIFUNGAL ACTIVITY OF LAWSON AND INDIGO COMPOUNDS

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ABSTRACT
Lowsonia inermis commonly known as" **HENNA**" belongs to the family Litheracae. Henna is widely used as herbal medical all over the world since centuries as an antifungul 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 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. 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. Antifungul effects of water, methanol extracts of L.inermis leaves and Indigo dye against clinical fungal cultures were investigated and compared with standard antibiotics of Ciprofloxacin and Ampicillin. The fungul isolates were treated with L.inermis methanol extracts were more effective than the water extract using standard method of NCCL, 2000.

KEYWORDS: *Lawsonia inermis*, *Indigofera tinctoria* , Extraction , Antifungal activity, Herbal Medicine.

INTRODUCTION

Nature is full of beautiful colors 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, mollusks 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, Cur cumin and Cochineal) (Mohd Yusuf et.al) The present study aimed at evaluating the natural antifungal 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 antifungal activity against Gram positive and against Gram negative fungi.

Natural dyes are used to impart color 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.CHENGAIAH* 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 lawson (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.CHENGAIAH* 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 color, 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)

EXPERIMENT AND 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. 1litr 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.

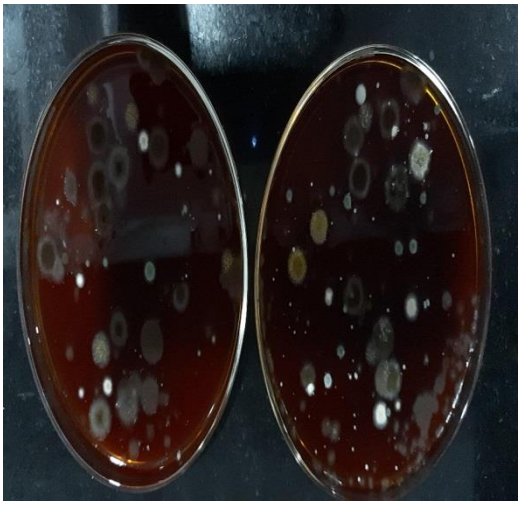

Fig. 2 – Drying the henna methanol extract.

INDIGO

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

Fig. 3 Indigo extract.

Antifungal activity

The antifungal activity was conducted to identify the activity of Indigo against fungus. Potato dextrose agar medium was prepared for the antifungal activity. This medium was left for solidifying for 24hrs. A negative control was also prepared in the same way using solvent DMSO. The disk diffusion method was carried by using two different fungal strains, they are *aspergilla's Niger* (black fungi) and *Trivhophyton mentagrophytes* (Tmen fungi). These strains were diffused into the media and incubated for 24hrs at room temperature in laminar airflow chamber. This results in the formation of zones or disks which eventually indicate the presence of antifungal properties, and if it is not present it shows no activity towards the fungi.

RESULTS AND DISCUSSION

Two different types of antimicrobial activity test were conducted on two different fungal 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 antifungal 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 antifungal activity and differing exists the size of the Ring indicates the degree of antifungal activity Agar dilution method is considered to be the reference method for their other antifungal 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 indigo and henna showed results of inhibition to the tested strains of fungi. The antifungal activity of Henna lowson compound and Indigo dye was as follows.

ANTIFUNGAL ACTIVITY

- Antifungal activities have been extensively studied. Antifungal activity to observe the growth inhibition and destroying of fungicidal and fungistatic. An antifungal medication also known as an antibiotic medication is a pharmaceutical fungi side or fungus static used to treat various diseases.
- The results showed that Tmen fungi showed the maximum results compared to aspergilla's Niger. The maximum diameter was obtained to Tmen fungi 15mm in Indigo and 14 mm in henna compounds.

Tab: 1 - Antifungal activity results.

Indigo	**1000mg/ml**	**500mg/ml**	**250mg/ml**	**Antibody**
Aspergillus Niger	10mm	8mm	7mm	
Tmen fungi	***15mm***	13mm	12mm	30mm
Henna water	**1000mg/ml**	**500mg/ml**	**250mg/ml**	**Antibody**
Aspergillus Niger	10mm	12mm	12mm	31mm
Tmen fungi	11mm	13mm	*14mm*	31mm
Henna	**1000mg/ml**	**500mg/ml**	**250mg/ml**	**Antibody**
Aspergillus Niger	11mm	12mm	13mm	37mm
Tmen fungi	10mm	9mm	8mm	31mm

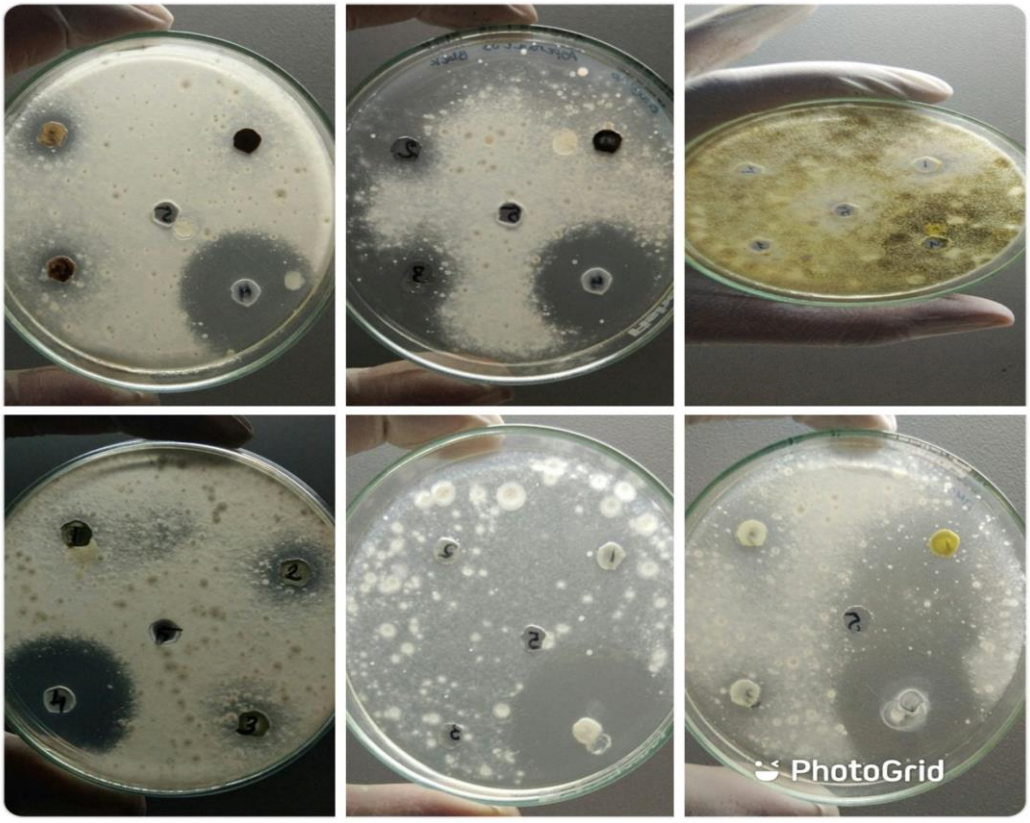

Fig: 4 Antifungal activity of Henna and Indigo dyes.

CONCLUSION

The present work shows that plant extract such as *Lawsone* and *Indigo* dyes were active against Aspergillus Niger and Tmen fungi that are pathogenic to humans where can be inhabited by lawsone and Indigo compounds. Indicant showed antifungal activities in the tested strains. Some of the tested strain show significant differences in their responses to the extracts by disk diffusion method. Primary vision caused by the extract of Indigo and Henna was observed partially, therefore it can be hypothesized that the basic dye of Indigo, Henna contain a wide spectrum of antifungal compounds with maximum 15mm diameter in the media.

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