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Research Article

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Synthesis, Characterization and Evaluation of Herbo-Mineral Complexes as Anti-Bacterial agents

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ABSTRACT

It has been postulated that the development of resistance to known anti-bacterials could be overcome by identifying new drugs. Hence, the objective of the work is to improve upon the existing anti-bacterial activity of the herbal drug molecule. The main purpose is to synthesize and characterize herbo-mineral complexes and evaluate them for anti-bacterial activity. This research study deals with synthesis of lawsone-copper, p-coumaric acid-copper, lawsone-zinc and p-coumaric acid-zinc complexes and their evaluation for anti-bacterial activity. The two herbal drugs viz. lawsone and p-Coumaric Acid were complexed with Zinc and Copper metals to form their respective complexes. The title compounds were synthesized, purified and characterized and screened against gram negative and gram positive bacteria. It can be concluded that such complexes can be utilized as novel anti-bacterial agents and check their anti-bacterial efficacy.

Keywords: Herbo-mineral complex, lawsone, *p*-Coumaric acid, anti-bacterial

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INTRODUCTION

Henna or Lawsoniainermis, containing a red-orange pigment and the molecule of which is also called hennotannic acid, when crushed in an acidic medium and applied to skin, the lawsone molecules migrate from the henna paste, traverse the outermost layer of the skin, Stratum corneum and stain the skin. Prolonged applications of henna result in diffusion of the pigment deeply into the skin. Chemically, lawsone is 2-hydroxy-1, 4-naphthoquinone. The name and molecular structure of henna show its congeniality to naphthalene. In lawsone, two oxygen atoms are attached to the naphthalene carbons at positions 1 and 4 to form 1,4-naphoquinone and a hydroxyl (–OH) group is present at position 2. Its molecule contains 10 carbon, 6 hydrogen and 3 oxygen ($C_{10}H_6O_3$), giving a total molecular weight of 174.16 amu. Pure lawsone is an orange powder, insoluble in water, with a melting point higher than 192ºC. [1] [2]

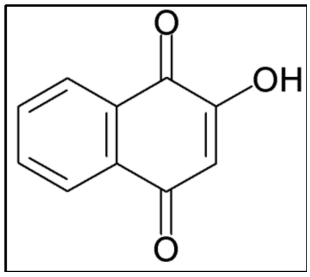

Figure 01: Structure of Lawsone

Coumaric acid is a hydroxy derivative of cinnamic acid and naturally occurs in three isomers (ortho-, meta- and para-); *p*-coumaric acid is one of the most commonly occurring isomer in nature. *p*-coumaric acid (4-hydroxy-cinnamic acid), classified as a phytochemical and nutraceutical, is found in various edible plants, such as carrots, tomatoes and cereals. *p*-coumaric acid (4-hydroxy-cinnamic acid) occurs widely in the cell walls of graminaceous plants [3]. It decreases low-density lipoprotein (LDL) peroxidation, shows antioxidant and antimicrobial activities and plays an important role in human health. It is found in the endosperm of kernels at a limited level; however, the amount of *p*-coumaric acid increases significantly in peripheral tissues. In terms of cereal types, it appears that pericarp fractions in barley, wheat, oat and corn are the fractions richest in *p*-coumaric acid. It is both a good antioxidant and a good antimicrobial; therefore, it is natural alternative instead of synthetic additives, nowadays.[4]

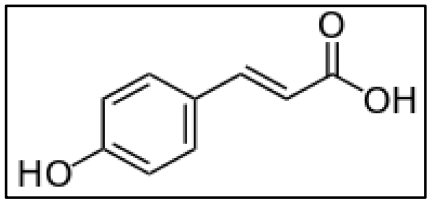

Figure 02: Structure of *p*-Coumaric Acid

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other new drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent.

EXPERIMENTAL SECTION

CHEMICALS

Lawsone was procured from Sigma-Aldrich Corporation Ltd., *p*-Coumaric Acid was procured from Alfa-Aesar, Zinc Acetate and Copper Acetate from Qualigens, Ethanol from the Government of Maharashtra and Ammonia solution from Loba Chemie.

PREPARATION OF HERBO-MINERAL COMPLEXES:

The selected drugs (1mol) and metal salts (1mol) were dissolved in ethanol and methanol separately. The two were then mixed together. The reaction mixture was stirred at room temperature with simultaneous adjustment to alkaline pH using ammonia solution. The mixture was then refluxed at 60˚C for 3 hours with intermittently adjusting the pH to alkaline. The reaction mixture was allowed to stand overnight. The precipitated complexes were filtered off, washed with solvent and finally dried in oven [8].

Metal salts used were Copper acetate and Zinc acetate separately for synthesis of Herbo-Mineral complexes.

CHARACTERIZATION OF HERBO-MINERAL COMPLEXES:

Infra-red spectra of title compounds were recorded in the range of 4000-400 cm^{-1} using KBR pellet method.

Ultraviolet (UV) spectra were recorded Perkin Elmer spectrophotometer. Thermo S-series Atomic Absorption Spectrometer was used to determine metal content. Karl fisher titrimeter was utilized for the determination of moisture content (Lab India Limited). Differential Scanning Colorimetry analysis was performed using Mettler Analyser.

1) MELTING POINTS AND PERCENT YIELDS

Table 01:

SR.NO	TITLE COMPOUNDS	MELTING POINTS (DECOMPOSITION) (°C)	PERCENTAGE YIELD
1	Lawsone-Copper complex	302	64.97
2	Lawsone-Zinc Complex	212	48.38
3	*p*-Coumaric Acid-Copper Complex	280	68.97
4	*p*-Coumaric-Zinc Complex	340	63.94

2)ULTRAVIOLET SPECTRAL ANALYSIS:

Ultraviolet (UV) spectra were efficiently recorded using Perkin Elmer spectrophotometer.

Table02:

SR.NO	TITLE COMPOUNDS	λ_{max}(nm)
1	Lawsone-Copper complex	290
2	Lawsone-Zinc Complex	460
3	p-Coumaric Acid-Copper Complex	285
4	p-Coumaric-Zinc Complex	285

3)INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY(ICP-AES):

Table 03:

Sample Code	Complex	% Free Metal
Zinc-01	Lawsone-Zinc Complex	25.63
Zinc-02	P-Coumaric-Zinc Complex	24.16
Copper-01	Lawsone-Copper Complex	23.1
Copper-02	P-Coumaric Acid-Copper Complex	23.19

4)KARL –FISHER TITRIMETRY FOR WATER CONTENT:

Karl – Fisher titration was performed to determine the water content. Karl – Fisher reagent was calibrated with disodium tartrate AR. An accurately weighed amount of compound was added to the dry methanol AR in KF reaction vessel and titrated against Karl – Fisher reagents. Titration was performed in triplicate to obtain reproducible results.

EVALUATION OF ANTIBACTERIAL ACTIVITY:

The antibacterial activity of title compounds was determined by agar diffusion method. Samples were initially dissolved in Dimethyl Sulfoxide at concentration-50ppm, 100ppm, 500ppm and 1000ppm. Isolates were grown for 24 h in nutrient broth to provide a turbidity of approximately 10^8cfu/ml. Bacterial suspensions were diluted with soft agar containing tubes at 45–50°C. These soft agar tubes were then poured over the agar plates previously prepared and allowed to solidify under laminar flow for 15 min. Bores of 9mm were produced using a borer. Sterile pipettes(100ul) were used in aseptic conditions to add the compounds into the bores. Same volume of the control (Dimethyl Sulfoxide) was also added on one of the other bores in each plate. The plates were then placed in an incubator at 37°C within 15 min of addition of the compounds into the bores. After 18–24 h of incubation, the plates were examined and the diameter of zones of complete inhibition was measured.

RESULTS AND DISCUSSION

UV spectral analysis of all synthesized complexes showed a shift as compared to parent drugs- Lawsone and *p*-Coumaric Acid.

IR spectral data showed prominent absorption peaks for peculiar functional groups at the expected frequencies for all title compounds.

Karl Fischer analysis for synthesized complexes showed small percentage of moisture which indicated the presence of coordinated water molecule with active molecule.

Inductively coupled plasma emission spectroscopy was carried out to determine the percentage of freemetal in synthesized complexes and was found to be satisfactory.

Differential scanning calorimetry, (DSC) was carried out to determine the degradation behavior of title compounds. DSC spectra of all synthesized complexes showed the degradation behavior and also showed absence of parent drug peak. These Spectra also indicated absence of melting point peak for all synthesized complexes. It can be concluded that such complexes may be utilized as novel anti-bacterial agents. Thus, it creates a future scope to carry out detailed microbial and toxicological studies. Also, similar such compounds can be prepared and studied so as to improve their antimicrobial spectra.

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