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BIOTRANSFORMATION OF DRUGS TO DRUG METABOLITES BY FUNGI ISOLATED FROM SOIL

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ABSTRACT

In this present study, the fungus was isolated from kollimalai hill soil sample by the method of serial dilution. The fungi were identified as *Aspergillus sp* by microscopic observation of lacto phenol red assay. The screening studies of microbial transformation were conducted for 10 different drugs. The aceclofenac, Atorvastatin and Pioglitazone showed the best conversion. Aceclofenac was taken for the preparative scale synthesis. *Aspergillus sp* was grown in higher quantity of media and aceclofenac was added. The crude compounds were extracted at 120 hrs and analyzed by TLC and HPLC. These results showed the maximum conversion of aceclofenac to other metabolites. The crude material was analysed by Mass spectroscopy for the studies of molecular weight determination of formed products. The soil microorganisms can be utilized for the microbial transformation of drugs. This method is the cost effective method and high yield product development. These compounds can be utilized for the pharmaceutical, toxicological and clinical trials. In future, the identification and characterization of unidentified compound will be determined.

KEYWORDS: Aceclofenac, Drugs.

INTRODUCTION

The soil is considered as the land surface of the earth which provides the substratum for plant and animal life. The soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa.

The physical structure, aeration, water holding capacity and availability of nutrients are determined by the mineral constituents of soil, which are formed by the weathering of rock and the degradative metabolic activities of the soil microorganisms. Cultivated soil has relatively more population of microorganisms than the fallow land, and the soils rich in organic matter contain much more population than sandy and eroded soils. Microbes in the soil are important to us in maintaining soil fertility, cycling of nutrient elements in the biosphere and sources of industrial products such as enzymes, antibiotics, vitamins, hormones, organic acids etc. But certain microbes in the soil are the causal agents of various human and plant diseases.

More than hundreds of different species of fungi inhabit the soil. They prefer to live in the soil in an aerobic condition. Fungi perform important functions within the soil in relation to nutrient cycling, disease suppression and water dynamics, all of which help plants become healthier and more vigorous. Fungi exist in both the mycelial and spore stage. Soil fungi are microscopic plant-like cells that grow in long thread like structures or hyphae that make a mass called **mycelium**. The mycelium absorbs nutrients from the roots it has colonized, surface organic matter or the soil. From the mycelia the fungi is able to throw up its fruiting bodies, the visible part above the soil (e.g., mushrooms), which may contain millions of spores. When the fruiting body bursts, these spores are dispersed through the air to settle in fresh environments, and are able to lie dormant for up to years until the right conditions for their activation arise. The physical structure of soil is improved by the accumulation of mold mycelium within it.

Fungi are active in decomposing the major constituents of plant tissues namely cellulose, lignin and pectin**.** Saprophytic fungi convert dead organic matter into fungal biomass, carbon dioxide and organic acids. These fungi have enzymes that work to "rot" or "digest" the cellulose and lignin found in the organic matter, with the lignin being an important source of carbon for many organisms. Without their digestive activities, organic material would continue to accumulate until the forest became a huge rubbish dump of dead leaves and trees. By consuming the organic matter fungi play an important role in immobilizing and retaining nutrients in the soil.

Some fungi live in a mutually beneficial relationship with plants. Mycorrhizal fungi are perhaps the best known of the mutualists. Mycorrhizal fungi form a partnership mainly with trees but also with some plants, but rather then harming the tree, their presence significantly

increases the roots' effectiveness. In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in arbuscular mycorrhizal fungi (AMF), or extracellularly as in ectomycorrhizal fungi. Arbuscular mycorrhiza (VAM) are the most common form of mycorrhiza, especially in agricultural plant associations. This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates, such as glucose and sucrose. The carbohydrates are translocated from the leaves to root tissue and on to the plant's fungal partners. Plant roots alone may be incapable of taking up phosphate ions that are demineralized in soils with a basic pH. The mycelium of the mycorrhizal fungus can, however, access these phosphorus sources, and make them available to the plants they colonize.

Another group of fungi also known as pathogenic fungi (parasitic fungi), the second largest group present in the soil. This group includes the fungi genera *Verticillium*, *Phytophthora*, *Rhizoctonia* and *Pythium*. These group of fungi being parasitic on plants, draw all the nutrients from the plant and ultimately cause its death. Fungi tend to dominate over bacteria and actinomycetes in acid soils as they can tolerate a wide pH range. Fungi can survive in the soil for long periods even through periods of water deficit by living in dead plant roots and as spores or fragments of hyphae.

Functions/role of fungi

Fungi plays significant role in soils and plant nutrition.

1. They plays important role in the degradation / decomposition of cellulose, hemi cellulose, starch, pectin, lignin in the organic matter added to the soil.
2. Lignin which is resistant to decomposition by bacteria is mainly decomposed by fungi.
3. They also serve as food for bacteria.
4. Certain fungi belonging to sub-division Zygomycotina and Deuteromycotina are predaceous in nature and attack on protozoa &nematodes in soil and thus, maintain biological equilibrium in soil.
5. They also plays important role in soil aggregation and in the formation of humus.
6. Some soil fungi are parasitic and cause number of plant diseases such as wilts, root rots, damping-off and seedling blights eg. *Pythium*, *Phyiophlhora, Fusarium, Verticillium*etc.
7. Number of soil fungi forms mycorrhizal association with the roots of higher plants (symbiotic association of a fungus with the roots of a higher plant) and helps in

mobilization of soil phosphorus and nitrogen eg. *Glomus*, *Gigaspora*, *Aculospora*, (*Endomycorrhiza*) and *Amanita, Boletus, Entoloma, Lactarius*(*Ectomycorrhiza*).

Drug development research

Developing a drug from the lab to the market is a massive task and a challenge. From the pre-clinical stage onwards, great care is desired to advance the compounds. Synthesis of compounds similar to that of the parent drug in the clinical trial will be a boon for the drug discovery programme. Failure of drugs in the clinical trials will have to be supported by group of compounds, whose structure or properties are similar to that of the drug under clinical trials. Synthesized compound or materials have to go through biological assays, *in vitro* and *in vivo* drug metabolism, pharmacokinetic studies, pharmacological and toxicological studies. After performing all the studies, compounds are to be ranked for clinical trials, and the compound that satisfies all the chemical and biological assays will be further tested. In the event of failure, compounds with next best properties are to be tested. Hence, synthesis of compounds with structure and properties similar to that of the parent compound in the clinical trial is necessary. There are many instances where metabolites have been developed as a drug. For example, desloratadine (clarinex) and fexofenadine (Allegra) were first identified as metabolites, and developed as drugs, later. Microbial and fungal biotransformation was also used to produce metabolites.

Drug metabolism

The ability of humans to metabolize and clear drugs is a natural process that involves the same enzymatic pathways and transport systems that are utilized for normal metabolism of dietary constituents. Humans come into contact with scores of foreign chemicals or xenobiotics (substances foreign to the body) through exposure to environmental contaminants as well as in our diets. Fortunately, humans have developed a means to rapidly eliminate xenobiotics so they do not cause harm. Metabolism is the enzymatic conversion of one chemical compound into another. Drugs are considered xenobiotics and most are extensively metabolized in humans. It is therefore not surprising that animals utilize a means for disposing of human-made drugs that mimics the disposition of chemicals found in the diet. This capacity to metabolize xenobiotics, while mostly beneficial, has made development of drugs more time consuming and costly due in large part to

- ✓ Inter individual variations in the capacity of humans to metabolize drugs,
- ✓ drug-drug interactions, and

- ✓ Species differences in expression of enzymes that metabolize drugs.

Stages of drug metabolism

Metabolism is often divided into two phases of biochemical reaction – phase-I and phase-II. Some drugs may undergo just phase-I or just phase II metabolism, but more often, the drug will undergo phase-I and then phase-II sequentially.

a. Phase-I Metabolism

Xenobiotic metabolizing enzymes have historically been grouped into the phase-I reactions, in which enzymes carry out oxidation, reduction, or hydrolytic reactions, and phase-II reactions, in which enzymes form a conjugate of the substrate (the phase I product). The phase-I enzymes lead to the introduction of some functional groups, resulting in a modification of the drug, such that it now carries an –OH, -COOH, -SH, -O- or NH2 group.

b. Phase-II Metabolism

Phase II metabolism involves conjugation - that is, the attachment of an ionized group to the drug. These groups include glutathione, methyl or acetyl groups. These metabolic processes usually occur in the hepatocyte cytoplasm. The attachment of an ionized group makes the metabolite more water soluble. This facilitates excretion as well as decreasing pharmacological activity. In phase-II it is conjugated with either glycine or glucuronic acid forming a range of ionized metabolites that can then be excreted in the urine. Phase-II enzymes facilitate the elimination of drugs and the inactivation of electrophilic and potentially toxic metabolites produced by oxidation. While many phase-I reactions result in the biological inactivation of the drug, phase-II reactions produce a metabolite with improved water solubility and increased molecular weight, which serves to facilitate the elimination of the drug from the tissue.

Sites of drug metabolisms

Xenobiotic metabolizing enzymes are found in most tissues in the body with the highest levels located in the tissues of the gastrointestinal tract (liver, small and large intestines). Drugs that are orally administered, absorbed by the gut, and taken to the liver, can be extensively metabolized. Within the cell, xenobiotic-metabolizing enzymes are found in the intracellular membranes and in the cytosol. The phase-I, CYPs, FMOs, and EHs, and some phase-II conjugating enzymes, notably the UGTs, are all located in the endoplasmic reticulum of the cell. The liver is the major “metabolic clear house” for the endogenous

chemicals and xenobiotics. The liver hepatocytes contain all the necessary enzymes for the metabolism of drugs. The main enzymes involved in metabolism belong to the cytochrome P450 group. These are a large family of related enzymes present in the smooth endoplasmic reticulum of the cell.

Microbial biotransformations

Targeted application of microbial transformation emerged only after the 19th century. The fusion of two sciences namely, organic chemistry and microbiology was the driver of tremendous growth of this field. The technology of microbial transformation deals with harnessing the enzymes in microorganisms to catalyze useful reactions on organic compounds. Great advances have been made in terms of exploiting microorganisms for biotransformation. The use of microorganisms for the synthesis of antibiotics and steroidal hormones evolved into large-scale industrial processes. Microbial transformations make use of enzyme catalyzed reactions with living cells, typically exploiting single chemical reactions like oxidation, reduction, hydrolysis, and degradation, formation of C-C or C-hetero atom bonds. Some of the advantages in selecting microbial reactions as alternative or supplement to chemical synthesis are,

- Microbial reactions can be used to functionalize specific positions in the molecules which are not normally possible by chemical methods,
- Oxygen function or other substituent's can be introduced stereospecifically or region specifically.
- Several individual reactions can be combined in one microbial step,
- The conditions under which microbial reactions take place are mile; hence compounds that are sensitive to heat, acid and base become amenable to microbial transformation,
- In some cases, it is cheaper to use a microorganism for the preparation of organic compounds than to synthesize it chemically. Thus it is not surprising to note that a large number of antibiotics and several of the medicinally important steroids hormones are currently produced on a large scale by microbial processes.
- To introduce centers of chirality into optically inactive substrates, and
- To carry out optical resolutions of racemic mixtures.

Another feature of microbial transformations is its ability to imitate mammalian metabolism of drugs. Thus key intermediates or metabolites of drugs can be produced in adequate amounts rapidly. This enables structure determination of drug metabolites for use in

preclinical trials, toxicity studies and regulatory process. Microorganisms do not always form the same metabolites as mammals; nevertheless, they are good models of drug metabolism. This approach has found wide success that led Smith and Rosazza to coin the term "Microbial Models of Mammalian Metabolism", to describe the use of microbial transformation systems as tool to facilitate mammalian metabolic studies. In addition to all these Microbial model was developed as one of the *in vitro* model to overcome the disadvantages of other models. Nowadays microbial model is used as a complementary tool to mimic mammalian metabolism by reducing the usage of animals in the drug development process.

Advantages of microbial models for mammalian metabolisms

Over 60% drugs were attired due to problems associated with metabolism such as undesired PK, PD, and toxicity profile. Compared with traditional methods using liver microsomes, microbial transformation is much more productive and scalable in preparing drug metabolites and elucidating metabolic pathways for drug discovery and DMPK studies. Microbial biotransformations constitute an important alternative as models for drug metabolism study in mammalians and have been used for the industrial synthesis of chemicals with pharmaceutical purposes. Several microorganisms with unique biotransformation ability have been found by intensive screening and put in commercial applications. Microorganisms such as fungi, bacteria and yeast have been successfully used as *in vitro* models for the prediction of mammalian drug metabolism with successful applications. An advantage of using microbial systems lies in the ease with which milligram quantities of metabolites can be produced by large-scale fermentation and under milder conditions than those required by chemical systems. Sufficient quantities of metabolites could then be isolated and used for pharmacological, toxicological, and analytical testing in conventional animal models of human metabolism, as part of the drug development process. To achieve this, attention was focused on microbes with broad metabolic capabilities.

The key advantagesinclude:

1. Inexpensive, fast, one pot, easy scale up
2. Less animal use
3. Moreyield
4. >95% success rate in modeling phase I human and animal metabolites
5. Good predictors of mammalian metabolites

6. Broad reaction types:C-hydroxylation, X-dealkylation, X-oxidation, etc.
7. Over coming difficulties in traditional chemical methods for pharmacophore modifications.
8. Integrated with HT screening for synthesis of phase II metabolites

Identification and characterization of drug metabolites

Metabolite identification is crucial to the drug discovery process because it can be used to investigate the Phase-I metabolites that are likely to be formed *in vivo*, the differences between species in drug metabolism, the major circulating metabolites of an administered drug, Phase-I and Phase-II metabolic pathways, pharmacologically active or toxic metabolites and can also help to determine the effects of metabolizing enzyme inhibition and/or induction. Identification of metabolites usually involves several steps: separation, detection, obtaining structural Information. The separation of metabolites can be obtained by TLC, Preparative TLC, column chromatography, flash chromatography and preparative HPLC. The basic need of Identifying Metabolites are to determine the molecular weight using Mass spectroscopy or LC-MS, to get structure information using NMR, to identify the functional group spectrum using FT-IR and to check the purity using HPLC. Hence the present investigation carried to screen the biotransformation of drugs to drug metabolites by fungi isolated from soil.

MATERIALS AND METHODOLOGY

Sample Sources

Soil sample was collected from Kollimalai hills, Salem. All the Drugs were obtained from Refsyn biosciences Pvt.Ltd., Puducherry. All the chemicals and reagent used for the work analytical grade.

Isolation of microorganisms from soil

Growing of cultures on solid media

The Sabouraud dextrose agar and nutrient agar mediums were prepared and autoclaved at 120 ºC for 15 minutes. The dilutions were labeled from 10^{-1} to 10^{-5}. The initial dilution was prepared by adding 1gm of the sample with 9ml dilutions blank labelled $10^{-5.}$ The contents were mixed by rolling the back and forth between hands to obtain uniform distribution of organisms. From the first dilution, 1ml of the suspension was transferred while in mention to the dilution blank 10^{-2} in second test tube. This procedure was repeated up to 10^{-5} in five test tubes. Using every time a fresh sterile pipette. From the appropriate dilution [10^{-1} to 10^{-5}]

0.1ml of suspension was transferred while mention with the respective sterile pipettes to sterile petridishes. six plates for fungi which contains Sabouraud dextrose agar. And six plates for bacteria which contain Nutrient agar. The media was allowed to solidify. 0.1ml of the sample was pipetted out into the petriplates. The L-rod passed over flame with bent position of the rod pointing down wards to prevent the alcohol from the running down. The rod was cooled for 10-15 seconds and the cover of the petridish was removed the L-rod was made to touch gently on the surface of the agar media and was removed back forth for complete spread of the culture, the petridish and L-rod procedure was closed. In which the bacterial plates were incubated from incubator into 37°c for 24 hours and fungi plates were incubated in BOD incubator 2-3 days at 26.7c°. After incubation the bacterial plates does not have expected level of result. But fungi shows the expected level of growth. so fungal plates selected and further proceed for screening of transformation.

Characterization of microorganism

Identification of lacto phenol cotton blue test and Characterization of microorganism by Morphological studies

Screening studies of microbial transformation of drugs using isolated organism.

Inoculation of culture on broth

- 1000ml soy bean casein digest broth was prepared and each 60ml was transferred into 10 flasks separately.
- All the flasks were kept for autoclave at 120 lbs for 20 minutes and kept for pre - incubation to see any possible contamination.
- After pre- incubation the culture were homogenized using 0.9% saline and 1ml of each culture was added to their respective broth for each culture.
- All the 10 flasks were incubated in orbital shaker for 48 hrs at 120 rpm 28ºc to carry out the reaction.

Addition of drugs

After 48 hrs 5mg of drugs namely Aceclofenac, Abiratenone, Atorvastatin, Clozapin, Desvenlafaxin, Imatinib, Ritinovir, Quetinapine, Pioglitazone, Vancomycin were added to each 10 flasks separately and labeled properly.

The shaking was continued with same condition.

Identification of metabolites thin layer chromatography

- Every 24, 48, 72, 96, 110, hrs the reactions were monitored by thin layer chromatography[TLC].
- To monitor the reaction, 2ml of reaction mixture was transferred on screw cap tube under sterile condition and the pH was adjusted to 9.0 using sodium hydrogen carbonate and 3ml was of ethyl acetate was added.
- The sample was shaked vigorously and allowed to separate into two layers the organic layer was spotted on above 1cm of readymade TLC plate.
- The TLC plate was kept on TLC chamber which already contains the mobile phase of chloroform : methanol [9:1v/v]. After elution, the TLC plates were dried and visualized under uv light and iodine chamber to identify the formation of metabolites.
- A preparative scale fermentation of *Aspergillus* species utilized preparative scale microbial transformation of sufficient quantities of the Aceclofenac metabolites for further identification.

Preparative Scale Production of Aceclofenac Metabolites By *Aspergillus* Species

- For the preparative scale reaction, the isolated culture were allowed to grow on soy bean casein agar slant and kept for 5 days at 24ºc.

Inoculam Preparation And Addition of Aceclofenac

- The preparative scale microbial formation was performed twelve 250ml of first- stage flakes containing 20ml soybean casein broth were generated as described previously.
- The spores or/ mycelia of culture from SDA slants were transferred in 90ml of sterile saline solution and homogenized for 10 minutes.
- The 1ml of Inoculum was transferred to each 90ml of soybean broth flasks.

The cultures were kept on rotary shaker at 24°C with 120rpm for 48 hours incubation, 5mg of Aceclofenac was dissolved in 1ml of methanol was added to each flask [5mg/90ml].

Extraction of Aceclofenac Metabolites

- After 120 hours (5days) of reaction, the flasks were removed from the shaker and filtered through s filter paper to separate the cultures and filtrate.
- The filtrate was used for further extraction the filtrate was adjusted to pH 9.0 using 2N $NaHCO_3$.

- Then the filtrate was extracted with three volumes of ethyl acetate; the organic layer was separated from aqueous layer.
- The culture and the aqueous layer were kept for decontamination.
- The organic layer was given a brine wash and dried over anhydrous sodium sulphate and filtered.
- The organic layer was concentrated under Roteva and the crude sample was transferred into a pre weight vial and the crude weight was recorded.

Analysis of crude metabolites of aceclofenac

Analysis by TLC

- The crude material of aceclofenac was spotted on TLC and observed the metabolite formation. Every 24, 48, 72, 96, 110, hrs the reactions were monitored by thin layer chromatography [TLC].
- To monitor the reaction, 2ml of reaction mixture was transferred on screw cap tube under sterile condition and the pH was adjusted to 9.0 using sodium hydrogen carbonate and 3ml was of ethyl acetate was added.
- The sample was Shaked vigorously and allowed to separate into two layers the organic layer was spotted on above 1cm of readymade TLC plate.
- The TLC plate was kept on TLC chamber which already contains the mobile phase of chloroform : methanol [9:1v/v]. After elution, the TLC plates were dried and visualized under uv light and iodine chamber to identify the formation of metabolites.
- A preparative scale fermentation of *Aspergillus* species utilized preparative scale microbial transformation of sufficient quantities of the Aceclofenac metabolites for further identification.

Analysis by high performance liquid chromatography

The formed crude compounds of aceclofenac was analysed by HPLC method.

Chromatographic condition

Instrument	: Waters 510 HPLC pump and Waters 486 Tunable detector
Software	: Autochro 3000
Column	: Silica, C18, 250mm x 4.6mm, 5 micron. (Grace)
Mobile phase	: Acetonitrile : Water (60:40)
Flow rate	: 1 ml/min.

Injectionvolume : 20 µl.
Column temperature : Room temperature
Detector : UV 280 nm.
Runtime : 3.7 min.
Diluent : Mobile phase

The sample was injected from sample loading system. And result was analysed.

Characterization of purified compound using mass

Mass chromatogram

A **mass chromatogram** is a representation of mass spectrometry data as a chromatogram, where the x-axis represents and the y-axis represents signal intensity.[The source data contains mass information; however, it is not graphically represented in a mass chromatogram in favor of visualizing signal intensity versus time. The most common use of this data representation is when mass spectrometry is used in conjunction with some form of chromatography, such as in liquid chromatography-mass spectrometry or gas chromatography-mass spectrometry. In this case, the x-axis represents retention time, analogous to any other chromatogram. The y-axis represents signal intensity or relative signal intensity. There are many different types of metrics that this intensity may represent, depending on what information is extracted from each mass spectrum.

The fractions collected from Column separation was analyzed in MASS Spectroscopy for the determination of molecular weight conformation.

Antibacterial activity test

- Muller-Hinton agar was prepared and poured in the petriplate.
- The *E.coli* organisms was inoculate into the plate by lawn technique.
- The antibiotic disc were placed in the agar which is coated with the sample. Incubate the plate 24 hours in incubate at 37°c.
- The zone were compared.

RESULT AND DISCUSSION

The ability of humans to metabolize and clear drugs is a natural process that involves the same enzymatic pathways and transport systems that are utilized for normal metabolism of dietary constituents. Humans come into contact with scores of foreign chemicals or

xenobiotics (substances foreign to the body) through exposure to environmental contaminants as well as in our diets. Fortunately, humans have developed a means to rapidly eliminate xenobiotics so they do not cause harm. Metabolism is the enzymatic conversion of one chemical compound into another. Drugs are considered xenobiotics and most are extensively metabolized in humans.

Drug metabolism is generally considered as a detoxification process and it leads to formation of relatively polar substances that are easily excreted from the organism. An important factor in the evaluation of safety and efficacy of any drug is the knowledge about its metabolism. A better understanding of the metabolism of drug is essential to know about drug action, distribution, toxicity, excretion and storage in the body origin.

Biotransformation is defined as structural modifications of chemical molecules using biological systems. There are a number of in vitro and in vivo biotransformation techniques available to generate metabolites. The in vitro techniques include the use of subcellular fractions prepared from cells that mediate drug metabolism, intact cell-based systems, intact organs, and isolated enzymes. In vivo methods involve the use of biological fluids (plasma, bile, urine, etc.) obtained from laboratory animals or humans dosed with the parent molecule. Microbial methods can also be used to generate metabolites. **(Aberra *et al.*, 2004).**

In this present investigation the biotransformation of drugs to drug metabolites by *Aspergillus* isolated from soil. The bacterial plates does not have the expected level of result. But fungi shows the level, so fungal plates selected and further proceed for screening of transformation. Aspergilus fungal plates have selected for the further studies.

The extracts of soybean casein broth was used for the work it shows the clear aqeous extracts of the product. In Thin layer chromatography the aceclofenac was almost converted into 80-90% to other compounds. The more converted spot showed the same R_f value of aceclofenac, but it is different in iodine activity.

In HPLC analysis, the standard aceclofenac and 120 hrs of crude samples were analysed. The retention time of aceclofenac was 2.9. In crude samples four different impurities were obtained with the RT values of 1.9, 2.9, 3.4 and 5.3.

The maximum conversion of impurity 1 is 65%. The faint presence of aceclofenac was 2%. This showed the aceclofenac was converted to their metabolites by the fungal culture of

Aspergillus sp . HPLC analysis of Aceclofenac standard and HPLC analysis of Aceclofenac crude compounds of 120 hours.

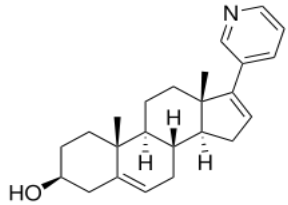
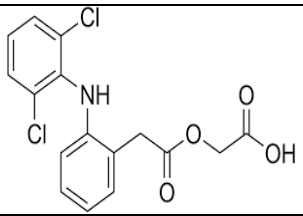
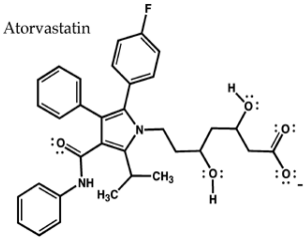
The fungal biotransformation of a Aceclofenac to the products 4-hydroxy aceclofenac, dichlofenac,4-hydroxy dichlofenac. In this screening it shows the major unknown peak of 389+1 was obtained.

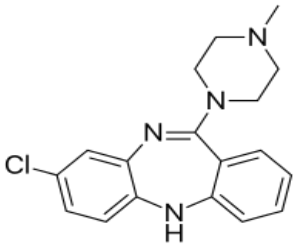
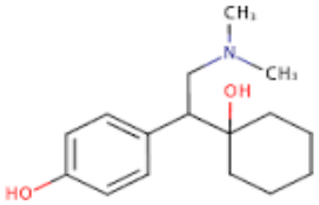
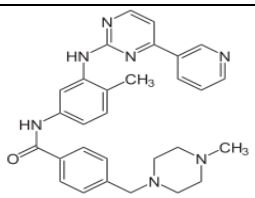
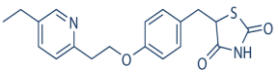
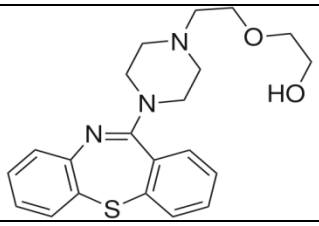
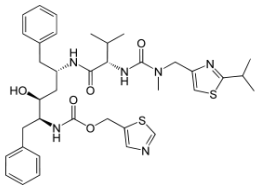
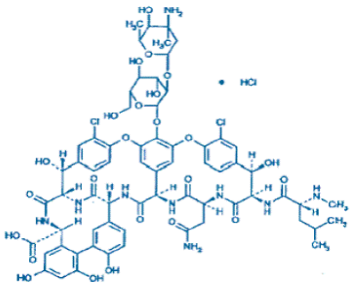
Antibacterial effect shows the 4-hydroxy aceclofenac,(8mm) diclofenac,(14mm) 4-hydroxy dichlofenac(12mm). Dichlofenac shows the better bioconversion of drug to drug metabolite. It shows the better zone formation when compare to other drugs. The drug metabolite have the antibacterial activity like the drug, but it does not have the side effect free the drug.

Soil borne organisms was used for screening it does not produce any harmness to the consumer. The biotransformation is the eco friendly process. these metabolites are used for reference standard in pharmaceutical studies, toxicological studies, and the new drug discovery.

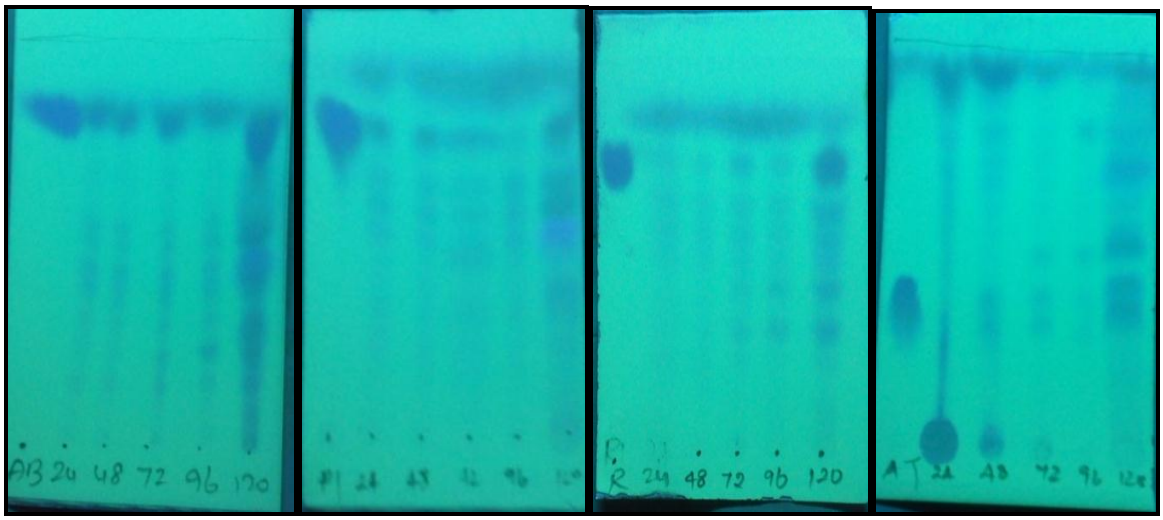
However the biotransformation of drug and drug metabolites need the further parametric studies to prove the drug metabolite as a novel drug discovery.

Drugs used in the study

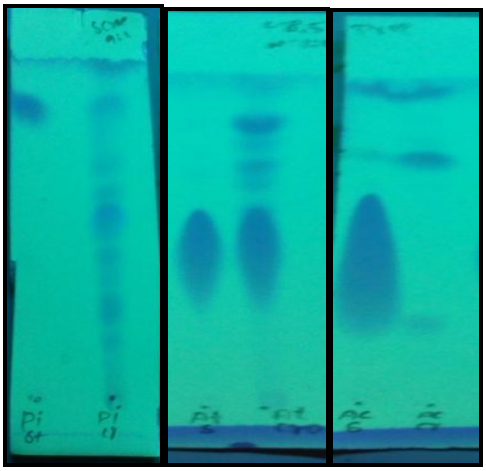
Drug name	Cas number	Molecular weight	Chemical formula	Mode of action	Chemical structure
Abiratenone	154229-19-3	349.509g/Mol	$C_{24}H_{31}NO$	Treat prostate cancer	
Aceclofenac	89796-99-6	354.18472 g/Mol	$C_{16}H_{13}Cl_2NO_4$	Anti-inflammatory drug	
Atrovastatin	134523-00-5	558.64g/Mol	$C_{33}H_{35}FN_2O_5$	Prevention of cardiovascular disease	

Clozapine	5786-21-0	326.823g/Mol	$C_{18}H_{19}C_1N_4$	Antipsychotic	
Dervanlafaxine	93413-62-8	263.38g/Mol	$C_{16}H_{25}NO_2$	Use in treatment is the major depressive disorders	
Imatinib	152459-95-5	493.603g/Mol	$C_{29}H_{31}N_7O$	Treat leukemia	
Pioglitazone	111025-46-8	356.44g/Mol	$C_{19}H_{20}N_2O_3S$	Control blood sugar levels	
Quetinapine	111974-69-7	383.5099g/Mol	$C_{21}H_{25}N_3O_2S$	Oral diabetes	
Ritinovir	155213-67-5	720.946g/Mol	$C_{37}H_{48}N_6O_5S_2$	Treat hiv/aids	
Vancomycin	1404-90-6	1449.3g/Mol	$C_{66}H_{25}NO_2$	Bacterial antibiotic used in resistant strains of *staphylococcus, streptococcus*	

Extraction of compounds from culture media



TLC screening of drug transformation from 24- 120hrs



Conversion of pioglitazone, Atorvasttain and aceclofenac

Antibacterial activity

A. 4-hydroxy aceclofenac. (8mm)

B. Dichlofenac. (14mm)

C.4-hydroxy dichlofenac. (12mm).

Rf values of positive compounds

Compound	Spots	Rf values	Visualization
Pioglitazone (0.8)	Spot-1 Spot-2	0.45 0.55	UV (254nm) UV (254nm)
Atorvastatin (0.56)	Spot-1 Spot-2	0.75 0.68	UV (254nm) UV (254nm)
Aceclofenac (0.5)	Spot-1 Spot-2	0.28 0.74	UV (254nm) UV (254nm)

Rf value: TLC analysis of crude compounds of aceclofenac

Crude compound of aceclofenac (0.4)	Spot-1 Spot-2	0.488 0.844	UV (254nm) UV (254nm)

HPLC Analysis of Aceclofenac Standard

S.NO	Name	RT (min)	Area (mV*sec)	Area %	Hight (mV)	Hight %	TP	TF
1	Impurities-1	2.0000	9.0647	6.25	0.6715	9.33	389.6	1.4191
2	Aceclofenac	3.7000	135.9656	93.75	6.5241	90.67	906.2	1.1486

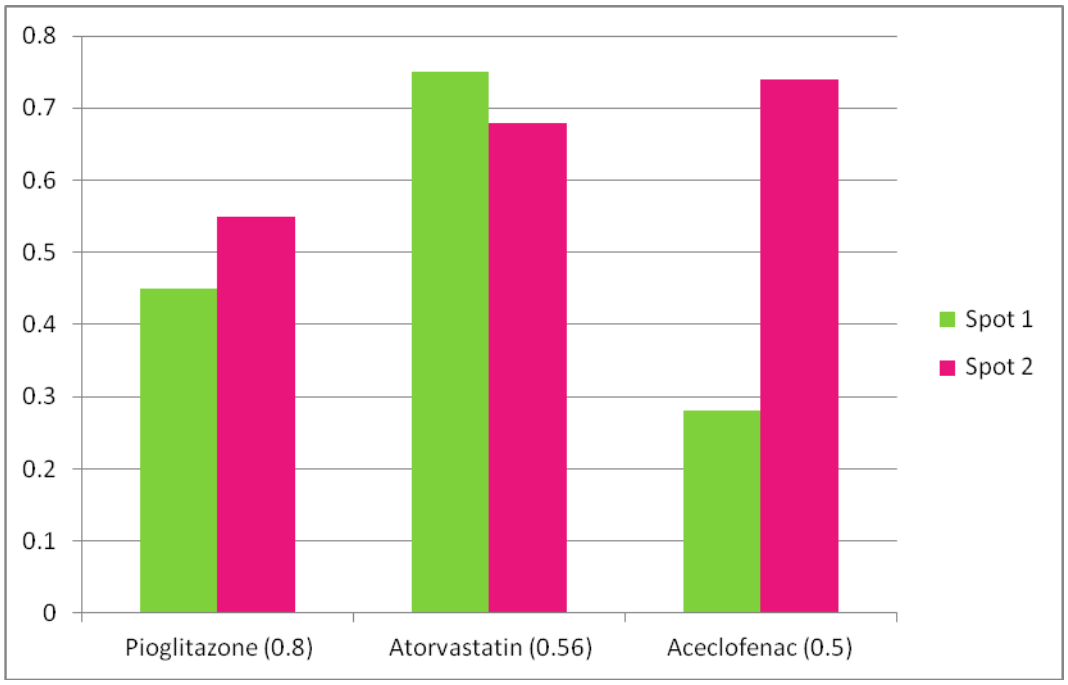
HPLC Analysis Of Aceclofenac Crude Compounds Of 120 Hrs

S.NO	Name	RT (min)	Area (mV*sec)	Area %	Hight (mV)	Hight %	TP	TF
s1	Impurities-1	1.9500	789.2408	64.58	72.63	72.63	539.4	1.7248
2	Impurities-2	2.9167	381.2762	31.20	24.02	24.02	362.2	1.2718
3	Impurities-3	3.4833	24.4165	2.00	1.83	1.83	1285.7	2.2739
4	Impurities-4	5.3000	27.2084	2.23	1.52	1.52	668.3	2.0434

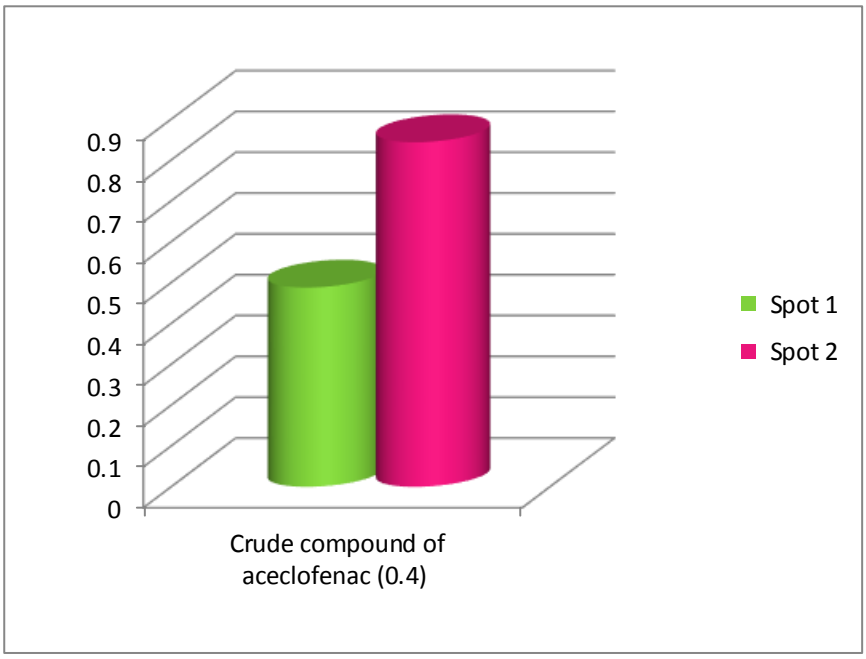
In Vitro Anti Microbial Activicty of Pathogenic Microorganisms

Microorganisms	Drug	Conc of drug	Zone of inhibition(mm)
E.coli	4-hydroxy aceclofenac	2.5mg	8 mm
E.coli	Dichlofenac	2.5mg	14 mm
E.coli	4-hydroxy dichlofenac	2.5mg	12 mm

Conversion of pioglitazone, Atrovastatine, Aceclofenac.



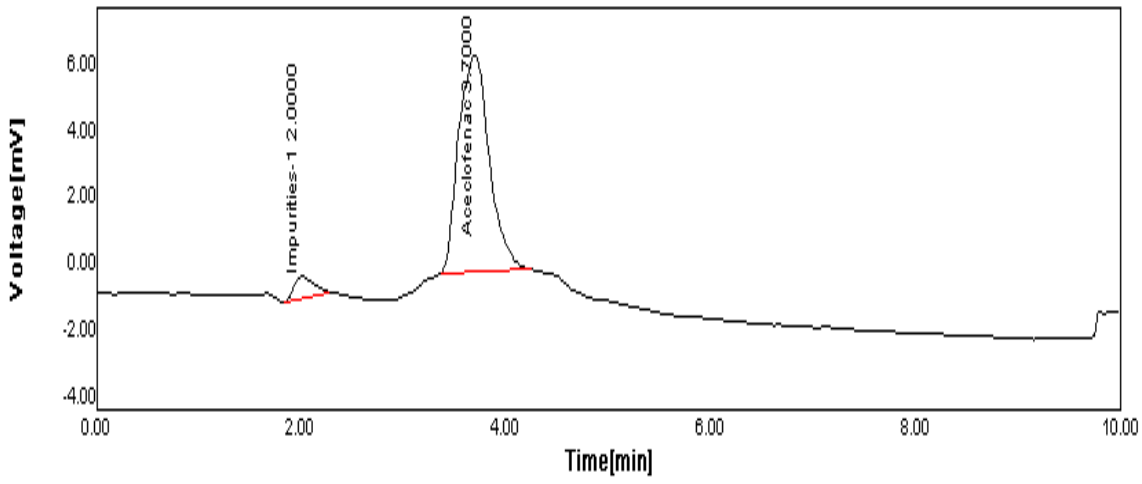
Rf value: TLC analysis of crude compounds of Aceclofenac



HPLC analysis of aceclofenac standard

Sample name : Aceclofenac standard

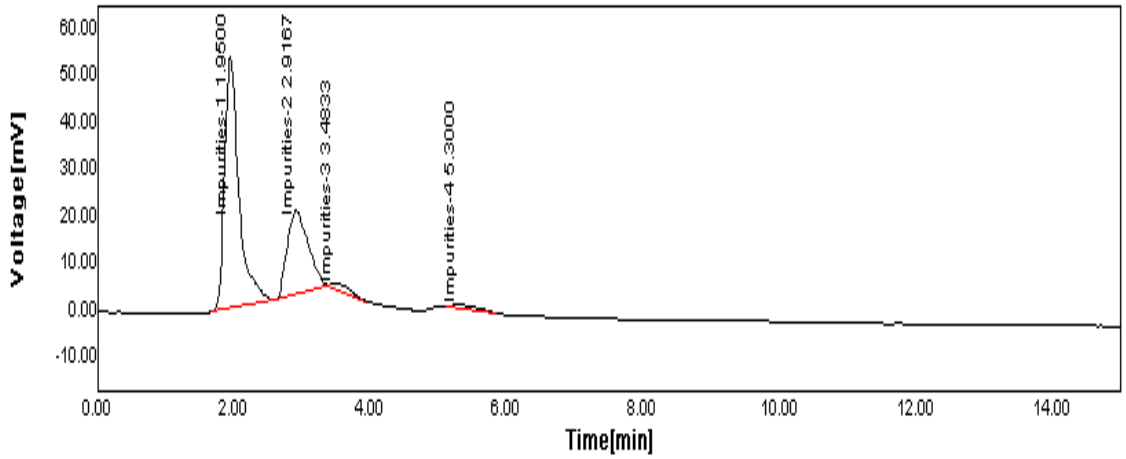
Sample id file : Aceclofenac 0001 raw



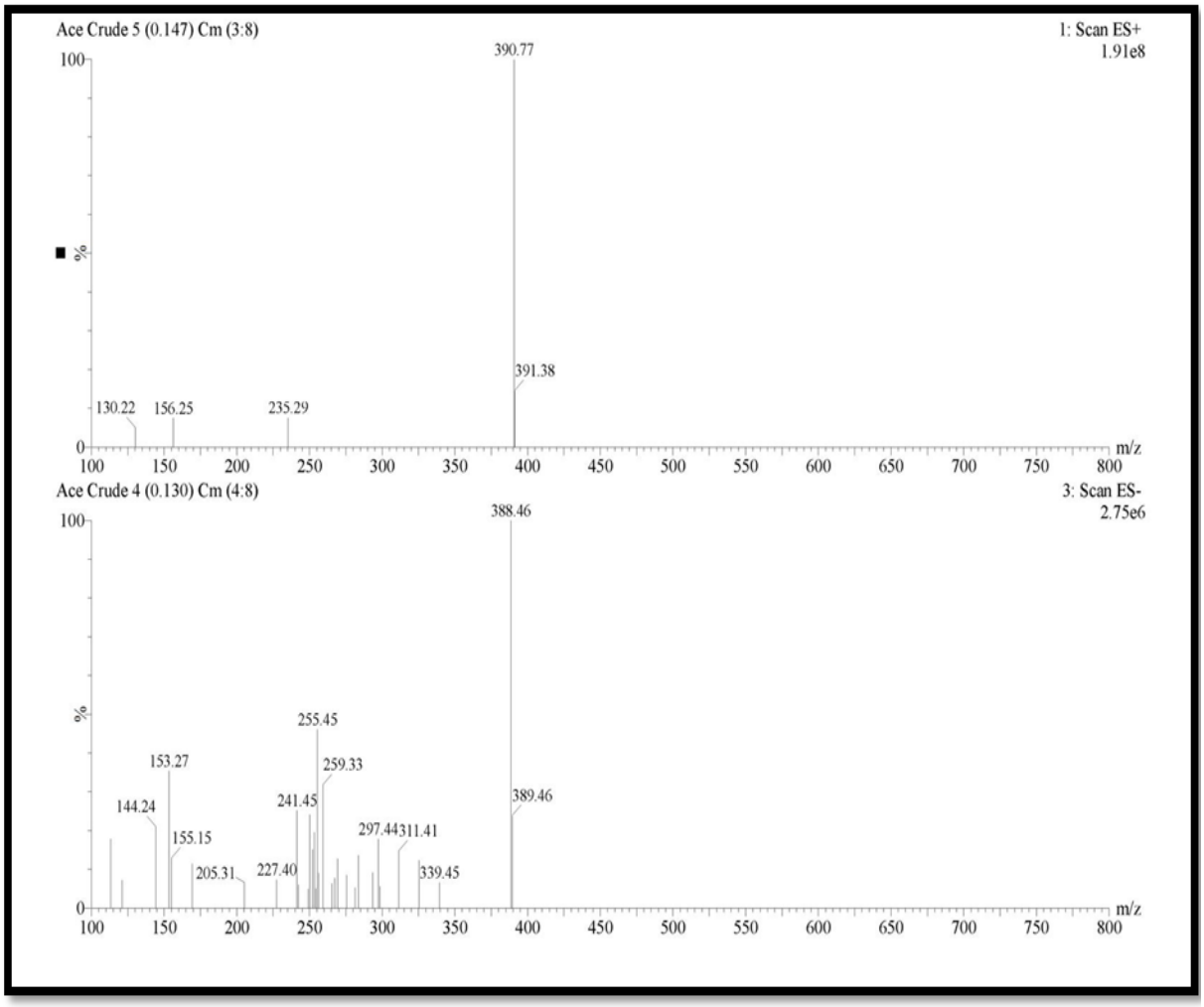
HPLC Analysis Of Aceclonofenac Crude

Sample name : Aceclofenac 120 hr crude

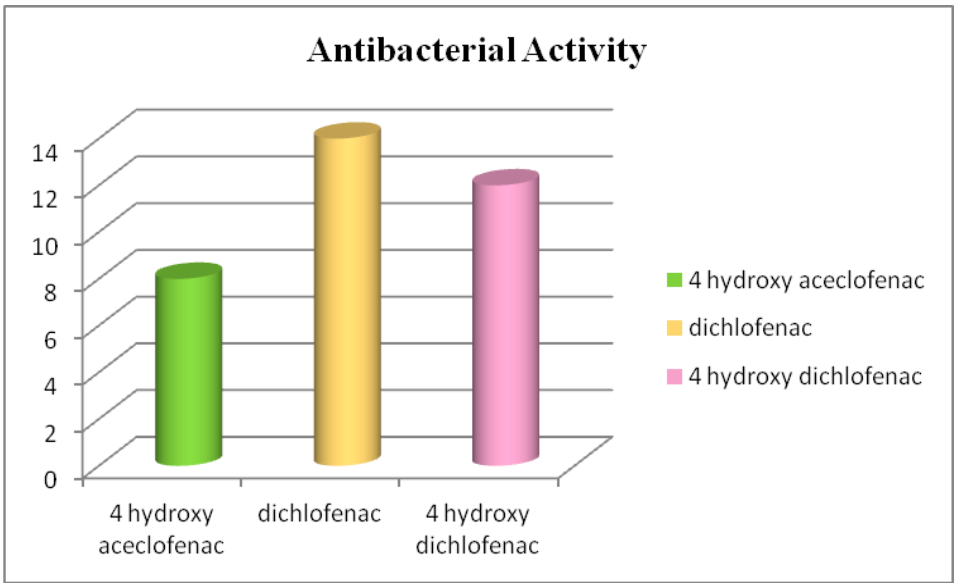
Sample id file : Aceclofenac 0002 raw



Mass Analysis of Crude Compounds of Aceclofenac By Soil *Aspergillus Sp*



Antibacterial Activity



CONCLUSION

The present study biotransformation aceclofenac to 4-hydroxy aceclofenac and dichlofenac to 4-hydroxy dichlofenac in an ecofriendly way. These metabolites can be used for reference standards in pharmaceutical studies, toxicological studies and for the new drug discovery. However a detailed parametric study is needed to produce polar metabolites in large quantities.

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