IOCBS Online Biology Conference 2017

IOCBS 2017

1st International Online Conference on
Biological Sciences
November 3-7, 2017
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INVITED PAPERS

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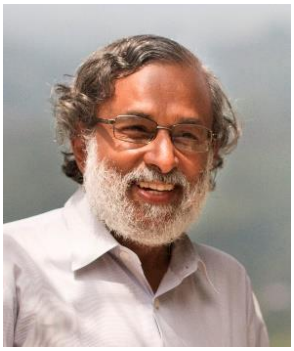
Key Notes

Biomedical Physics & Technology research targeting healthcare of the common people - *in the light of Bangladesh experience*

Dr. K Siddique-e Rabbani



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Abstract

While being a PhD student in UK working in Microelectronics, the author came to realise that the quality of life of the common people in those countries is high as their science and technology researchers addressed day to day problems of life, a process which is continuing till this day. On the other hand such efforts are completely absent in our countries branded as the 'Third World'. Although scientists and technologists had been doing research in these countries over many decades, no significant change in the life of the common people has happened so far. This is mainly because most of the science and technology researchers in the Third World, after getting higher education and training in the Western countries, continued the same research problems, which are mostly geared to the problems of those countries. Besides, publication of papers appears to be the prime target of these researchers. The target to address the problems of the common people in their own countries was absent.

This realisation made the author change his research topic after his return home in 1978. At home he looked for avenues in science that could be useful to the people and eventually became engaged in the field of Biomedical Physics & Technology. Since then he and his team of students developed several modern healthcare devices including computer based ones which are being used in hospitals, clinics or by patients, even beyond the borders of Bangladesh. Some were routinely used for more than twenty years, unimaginable for any imported equipment! These devices include, i) Computerised EMG/Nerve conduction

equipment, ii) Computerised ECG equipment (also used in telemedicine for online transmission of data), iii) Computerised dynamic Pedograph (for foot pressure distribution mapping), iv) Iontophoresis equipment for treatment of excessive sweating (used by patients at home), v) Muscle & Nerve stimulator (for physiotherapy), vi) Low cost semi-functional mechanical prosthetic hand, vii) Intraoperative Neuro Monitor (for monitoring nerves during brain or spinal surgery) and viii) Electrical Bio-Impedance device for localised physiological monitoring.

Such efforts also gave the expertise and experience to fill in gaps that were revealed when the devices were used for clinical work or for research, which led to innovations in the areas of nerve conduction in the form of a new parameter, 'Distribution of F-Latency (DFL)' and in general physiological study and diagnosis through another new technique, 'Focused Impedance Method (FIM)'. Both of these have been taken up for research by advanced universities of the world including Norway and UK. The author's team deliberately refrained from taking out patent, rather they have established an 'International Centre for Technology Equalisation (ICTEq)' for dissemination of the matured technologies. They have also innovated very simple techniques for providing germ-free drinking water using solar Pasteurisation that a rural user can make from easily available materials. The author's group also got involved in the manufacture and dissemination of the developed technology and in establishing a telemedicine network within the country developing their own hardware and software. The success of all the above effort has started to become visible and has been acclaimed nationally and internationally. All these efforts and models for dissemination have immense potentials to solve some of the major problems in the healthcare sector, particularly in the Third World.

Pharmacovigilance and Drug Safety: Practical difficulty and Challenges

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Introduction

Today's increasing pace of innovation in the Medical related industries is resulting in ever larger number of drugs and medical devices coming to the market every year. Despite the continued work during the past few decades towards harmonization of regulations across various regions, the fact remains that there are wide differences in regulatory bodies and the respective regulations they mandate on medical and pharmaceutical sectors functioning in their ambit. [1] As per global data adverse drug reactions are the sixth leading cause of death and the incidence of serious adverse drug reactions in hospitalized patients is almost 7%. This needs to address the different barriers along with challenges of pharmacovigilance and also requires necessary actions to be taken in order to deliver healthcare to the people of our motherland. [2]

Confronts in Pharmacovigilance science

Herbal medicines in India are vast spread and diversified. This range adds to the challenges of herbal pharmacovigilance including basic questions such as defining the most appropriate herb naming system (botanical, common, pharmaceutical name or herbal drug name) and validation of the botanical identity of the herbal ingredients.

- NPP encouraged reporting of all suspected ADRs, But number of reports related to Ayurvedic herbal drugs are abnormally low.
- Concept & terminologies related to ADR monitoring are not covered in the Ayurvedic curriculum.
- Methods to study drug safety problems have not evolved adequately in Ayurved.
- Information related to medicines is in the form of slokas in the texts, it is not easily available for general public.
- Signal detection is difficult because of inherent belief that Ayurvedic medicines are safe.
- Patients often use medicines from different systems of medicine concomitantly - difficulty in assigning causality.
- Lack of quality assurance and control in manufacture of Ayurvedic medicine.
- Most Ayurvedic formulations are multi-ingredient. [3, 4]

Pharmacovigilance need to continuously evolve their processes and systems to effectively support growth and ensure compliance. The point below summarizes the challenges faced:

Key Challenges

1. Continuously evolving regulations and business processes.
2. Necessity to maintain compliance at national and international levels.
3. Efficient and cost effective operations across all markets.
4. Large number of reporting requirements.
5. Signal detection and management.
6. Data Quality and Access.
7. Quality control and assurance procedures.
8. Maintain Compliance.
9. Manage global benefit-risk profile across entire product portfolio.
10. System Integration.
11. Application Reliability, Availability & Scalability.

12.System security & data privacy.

13.Vendor Responsiveness.[5]

Discussion & Conclusion:

A rigorous Pharmacovigilance program, incorporating and supported by all health professionals involved would eventually reassure the community regarding their safety. Adverse events can be brought to a minimum level by having sound knowledge about the side effect of the drugs. Improvement of communication regarding Pharmacovigilance between public and health professionals creates awareness and adverse occurring can be minimized. Proper knowledge on Pharmacovigilance would help to health professionals to understand the effectiveness or risk of medicines that they prescribe and ensure a better healthcare to patient and solving out the practical difficulties and challenges. Pharmacovigilance departments need to weigh multiple factors as they chart out their own roadmaps. Whether organizations decide to develop their PV applications in-house or procure them from a vendor, giving due weightage to each role in the PV team, the unique challenges they face and how they will evolve in the future can go a long way in establishing a sound strategy that serve will eventually serve the business well.

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Evaluation of Effect of Krishnadi Choorna in Management of Tamak Shwas W.S.R. to Bronchial Asthma

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Abstract

In the Current Study 60 Patients of Tamak Shwas have been selected randomly divided in two groups. The patients showing classical symptoms of Tamak Shwas such as Shwaskruchhrata (Dyspnoea), Kasa (Cough), Ghur-Ghurak Shabda (Wheezing or Rhonchi) During night, Kasten Shleshma Moksha (Difficult in Expectoration), Kasten Bhashya (Difficult in Expectoration), Anidra (Insomnia) etc. were included in this study. For the present study we were given Krishnadi Choorna orally. It reduces Respiratory Rate effectively & increases Expansion of Chest, Breath Holding Time, and Peak Expiratory Flow Rate and Sustained Maximal Inspiration which was highly significant statistically as compared with Tab. Deriphyllin.

Key words- Tamak Shwas, Krishnadi Choorna, Bronchial Asthma.

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ARTICLES

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Original Research Article

Association of MMP 8 and 12 Gene Polymorphisms with Chronic Periodontitis in Indian population

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Abstract

Background: Periodontitis, a common inflammatory oral disease, results damage of the surrounding cells and connective tissue structures, including alveolar bone, causing gum bleeding, tooth loss. Matrix metalloproteinases (MMPs) are related to tissue destruction and remodelling events in periodontal diseases. Single nucleotide polymorphism (SNPs) in MMP8 and MMP12 gene is associated with the risk of some inflammatory diseases. Therefore, the aim of this study was to investigate the association between MMP8(rs2155052) and MMP12(rs2276109) gene polymorphism and chronic periodontitis in an Indian population.

Materials and Methods: Genomic DNA was obtained from 60 chronic periodontitis patients and 60 healthy subjects. MMP8(rs2155052; +17 C/G) and MMP12(rs2276109; -82 A/G) gene polymorphism were amplified by polymerase chain reaction (PCR), and the polymorphisms were analysed by Sanger method of sequencing. We calculated the chi square with specific odds ratios along with their 95% confidence intervals to compare the allelic and genotypic distribution between cases and controls. Epidemiological factors like age, gender, oral habits etc. had been studied to find any impact on the occurrence of chronic periodontitis. All statistical analysis has been performed by SPSS software package version 16.0.

Results: In case of MMP8(+17C/G) gene polymorphism the distribution of the GG genotypes was lower in the chronic periodontitis patient (OR=0.53; 95%CI=0.04-5.99; p=0.53) than control groups. Whereas there was no difference in MMP12(–82A/G) polymorphism between CP and control group (OR=1.02; 95%CI=0.06-16.72; p=0.75). Smoking habit was also associated with increased risk of this disease (OR=8.5, 95% CI=6.73-15.81, p=<0.001) where chewing tobacco habit did not show any significant association.

Conclusion: Our results suggest that MMP8 and MMP12 gene polymorphism might not be associated with the susceptibility to chronic periodontitis though further study is needed to be carried out in larger population.

Keywords: MMP, SNP, chronic periodontitis, PCR, sequencing, chi square.

IOCBS Online Biology Conference 2017

Original Research Article

Study of Tumor Necrosis Factor – α (-1031C>T) rs1799964 Gene Polymorphism in Chronic Periodontitis patients of Eastern Indian Population

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Abstract

Background: Tumor necrosis factor-alpha (TNF-α) is a major mediator and a cell signalling protein, whic h get involved with the immune inflammatory response. It is a multifunctional cytokine which may help to study the progression of chronic periodontitis and some other related pathogenesis. It was also recognized that TNF-α is the prototypic member of a large cytokine family. And Polymorphisms in the promoter of th e TNF-α gene have been associated with some types of inflammatory diseases. The present study investig ated the association between a single nucleotide polymorphism (SNP) of the TNF-α (-1031C>T) gene and chronic periodontitis patients of Eastern Indian Population.

Methods: One Hundred and twenty subjects were recruited for the study: 60 healthy individuals (control g roup) and 60 patients with chronic periodontitis. Genomic DNA was obtained from peripheral blood. Amp lification reaction was performed for TNF-α (-1031C>T) gene polymorphism by using the already designe d primer in polymerase chain reaction and sequence were analyzed by Sanger method. Genotype and Alle le frequencies were calculated, and data were statistically analyzed using Chi-square test ($p<0.05$).

Results: There were a significant differences found in genotype frequencies (P=0.055, OR=0.4, 95%CI=0 .17-0.94) and allele frequencies (P=0.0138, OR=0.50, 95%CI=0.30-0.86) in TNF-α gene polymorphism o f TNF-α (-1031C>T) between patient and control groups susceptibility to chronic periodontitis. Logistic re gression analysis also shows that smoking is another significant risk factor for chronic periodontitis (P=<0 .001, OR=8.5, 95%CI=6.73-15.81).

Conclusion: TNF-α (-1031C>T) promoter gene polymorphism is associated and might contribute to the s usceptibility of chronic periodontitis in Eastern Indian population. A major number of samples may requir e or other polymorphism must be involved in determining susceptibility to that particular disease. Future r esearchers should try to strictly control and maintain various confounding factors to ensure the reliability o f the results with explicit inclusion and exclusion criteria of the cases within the study population for bette r conclusion.

Keywords- SNP, Genotype, Periodontal disease, TNF-α.

IOCBS Online Biology Conference 2017

Original Research Article

Study of Polymorphism in Interleukin 1 Beta Gene Associated with Chronic and Aggressive Periodontitis in Eastern Indian Population

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Abstract

Background: Periodontitis is a very common disease in middle and old age population but if not treated in time it can cause permanent teeth loss. It is a multifactorial disease where epidemiological factors like ethnicity, gender, food habits, addiction, etc, can be associated with it. Other factors like genetic factor has shown to be strong influence in different ethnicity of population. Many study has shown that Single nucleotide polymorphism (SNPs) in inflammatory genes has a strong association with periodontitis in different ethnicity of population. There are many forms of periodontitis but we chose chronic and aggressive periodontitis in our study as it is very common in population. The sole purpose of this study is to investigate the association and the frequency of IL1B (-3954 C/T, -511A/G, -31C/T) gene polymorphisms with susceptibility to chronic and aggressive periodontitis in eastern Indian population.

Methods: The study was designed as a case-control study that includes (N=60) with chronic periodontitis and (N=20) having aggressive periodontitis and the healthy group (N=60) included individual with no clinical signs of inflammations with mean age group of 40±10 years. A structured questionnaire was conducted to determine epidemiological factors. Genomic DNA was analyzed for polymorphism in the IL-1B gene at site -3954, -511, -31 by polymerase chain reaction (PCR) amplification followed by DNA sequencing. Data were analyzed by chi square test, analysis of variance (ANOVA), and by calculating odds ratio (OR) and 95% confidence intervals (CI).

Results and discussion: The regression analysis for epidemiological factors shows that tobacco smoking has a strong association with periodontitis. In the case of IL1B (-511A/G) AG genotype frequency (P=0.04) showed significance in chronic periodontitis patients. In case of IL1B(-3954 C/T) TT genotype frequency (P=0.032) and T allele frequency(P=0.032) for and significantly associated to aggressive periodontitis. As it obeys the logistic regression of(P=<0.05) and hence they are significant.

Conclusion: Our study shows that IL1B (-511A/G) and IL1B (-3954 C/T) could be a risk factor for chronic and aggressive periodontitis respectively while IL1B(-31C/T) shows no association compared to healthy subjects in Eastern Indian Population.

Keywords-Gene polymorphism, Chronic Periodontitis(CP), Aggressive periodontitis(AP), Epidemiological factors

Hemolymph (HL) miRNA: A Pill Controlling Aging

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Abstract

Objective: To show that the miRNAs circulating in the hemolymph (HL) of *Drosophila* is in complete correlation with the miRNAs circulating in the body fluids of mammals (for example, Humans) thereby regulating aging.

Material and methods: Fresh *Drosophila* stocks were used to obtain hemolymph (HL) and body tissue (BT). Qiagen miRNeasy Serum/Plasma kit was used to obtain HL-miRNA and BT-miRNA. Real-Time PCR was done to quantify cellular mRNA and reverse transcribed copy of HL-miRNA and BT-miRNA i.e., cDNA. Subsequently, amplified cDNAs were subjected to Gel Electrophoresis to validate the size thereby constructing a small RNA library. Lastly, stability of miRNAs was performed by examining their susceptibility to nucleases (RNase A and DNase I).

Results: Both Hemolymph (HL) miRNA and human miRNAs circulate in vivo in stable form and are nuclease resistant. Both *Drosophila* and human miRNAs are similar in function. Homologs of *Drosophila* hemolymph (HL) miRNAs are present in human body. Among them, miRNA184 is an important one.

Discussions: The miRNA184 regulates lifespan in both *Drosophila* and humans through IIS (Insulin/Insulin like growth factor pathway). Due to activation of this pathway, Akt/PKB gets activated. The phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway, then leads to the inhibition of the forkhead box O (FOXO) transcription factors. Another conserved target of Akt is the target of rapamycin complex 1 (TORC1) which is indirectly activated by Akt. To mediate autophagy, several autophagy specific genes are required that codes for autophagy related proteins. These genes like Atg8a (in Drosophila) and LC3 (Microtubule Associated Protein 1A/1B-Light Chain 3) in humans, homolog of Drosophila Atg8a, plays an important role to carryout autophagy. The miRNA184 leads to activation of these autophagy specific genes thereby increasing longevity. Similarly, if these genes remain inactivated due to reduced expression of miRNA184, then it leads to impaired autophagy that leads to aging. In Drosophila, overexpression of miRNA184 due to activation of FOXO transcription factor, leads to decrease in IIS pathway, leading to decrease in mTOR. As a result, Atg8 gets activated to carryout autophagy that leads to

increased longevity. In human also, overexpression of miRNA184 leads to reduction in the amount of its target proteins (in this case it is Akt2). Thus, proper autophagy in both *Drosophila* and humans increases longevity. Similarly, reduced expression of miRNA184 leads to inactivation of autophagy specific genes leading to impaired autophagy that further leads to reduced longevity. This impaired autophagy is mainly responsible for Epilepsy in children that show congenital aging producing aged phenotype at an early age.

Conclusion: Both mammalian and HL- miRNA is present in an age dependent manner mediating intercellular communication thereby regulating aging /lifespan. These HL miRNAs can be used as a biomarker to control/monitor the pathways regulating aging. These HL-miRNAs can be used in the study of disease related research of humans.

Keywords- Drosophila, hemolymph microRNA, humans, aging and biomarker

GC-MS Analysis of Bioactive Compounds Present in Methanol Extract of Fruit of An Endemic Plant Kayea assamica

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Abstract

Kayea assamica is an endemic plant species belongs to the family Clusiaceae found only in Padumoni Park and Dullung Reserved Forest of Lakhimpur district of Assam, India (27°24'39.6"N 94°11'32.0"E). It is a slow growing tall ever-green tree of 20-25 m height and the local people use it mainly as firewood due to lack of awareness about its other importance. Its population is gradually decreasing in that particular habitat. Locally it is known as "Sia-nahor" and *K. assamica* is a synonym of *Mesua assamica* (King& Prain). The fruits of *K. assamica* are used as fish poisoning in that particular region by the local people for fish catching in river and ponds and the stem bark also used in treatment of malaria by the ethnic people. It has well ethnopharmacological evidence, but don't have any scientific validation. The objective of our study is to investigate and characterize the chemical composition of the methanol crude extract of fruits of *K. assamica*. The fruits were cut into small pieces and subjected to methanol extraction, then the extract was further subjected to Gas chromatography-Mass spectrometry (GC-MS) analysis. The result revealed 13 different compounds which are copaene, β-farnesene, β-bisabolene, δ-cadinene, alpha.-bisabolol, cetyl alcohol, cetrimonium bromide, palmatic acid, 5,12-naphthacenedione, 8-acetyl-1,6,10,11-tetrahydroxy-, 6.beta.bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.a, d-homo-24-nor-17-oxachola-20,22-diene-3,16-dione, 1,2:14,15:21,23-triepox, 5,12a-diazaindeno[1,2-a]phenalene-3-carbonitrile, 12(12ah)-oxo- 295, and apometzgerin**.** These chemical compounds reported to have important bioactivities including antioxidant, antimicrobial, aphicidal, cytotoxic, anti-ulcer, larvicidal, antiproliferative, apoptotic, *in-vivo* wound-healing, anti-cancer, anti-infective, self-cleaning and bioremediation in oily soil, hypocholesterolemic, nematocidal etc. Many of the identified compounds are recognized as major constituent of essential oil producing plants. Out of these identified compounds, β-farnesene is a terpene, which has lervicidal effect against six mosquito vectors and acute toxicity on non-target aquatic organisms, due to which it can be predict that β-farnesene is responsible for the fish poisoning. Beside these activities, β-farnesene has potential aphid control activity in agricultural field and precursor of fuel. Other compounds like δ-cadinene is a novel larvicide against Malaria, Dengue and Filariasis mosquitoes and has repellent activity, copaene has anti-genotoxic activity, alpha.-bisabolol has anticancer, anti-infective and wound-healing activity, cetyl alcohol

has anti-proliferative effect (chemopreventive potential) against human cervical cancer cells HeLa, it also has antioxidant, anti-inflammatory and efficient bioremediation activities, cetrimonium bromide have significant cytotoxic, antifungal and anti-protozoal activities; the central mode of cytotoxicity of cetrimonium bromide is mitochondria-mediated apoptosis. Another important compound apometzgerin has strong inhibitory activity against alpha-glucosidase *in vitro*, that indicate the possibility of use in treatment of diabetes. Thus, identification of different biologically as well as pharmacologically active compounds in the fruit extract of this endemic plant warrants further studies on it and advised for conservation of it.

Keywords- *Kayea assamica*, Endemic, Bioactive compounds, GC-MS.

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Original Research Article

Delimitation of Species of Family Lymantriidae (Lepidoptera: Noctuoidea) Using External Genitalic Attributes

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Abstract

"Species are groups of interbreeding natural populations that are reproductively isolated from other such groups."(Mayr, 1940). Thus species has three separate functions: it forms a reproductive community, an ecological unit and a genetical unit. To discover and describe species is one of the major goals of systematics. Species delimitation is the process by which species boundaries are determined and new species are discovered. Generally species are limited, based on one or more qualitative and quantitative morphological characters that are not overlapping with other species. Genital morphology is that characteristic feature that defines an insect species based on inter and intra- specific genital variations. Genitalia are generally complex and very diverse. In order to distinguish between the closely related species, the external genitalia of male and female serve as a significant tool. In insects, the structures of male and female genitalia are important as they are highly species specific. Many parts of external male genitalia (uncus, gnathos, valvae, vinculum, aedeagus and its armature) are original modifications of dorsal and ventral plates of segments A9 and A10 (Scoble, 1995). The female genitalia are of the usual Ditrysian type with papilla analis, anterior and posterior apophyses, ductus bursae, corpus bursae and modified signa. New methods for species delimitation are being developed including DNA barcoding (Herbert *et al.*, 2003, 2004), DNA taxonomy (Tautz *et al.*, 2003) and Web based taxonomy (Godfray, 2002, Scoble, 2004, Knapp *et al.*, 2007). Although study of genitalic attributes for species delimitation is traditional but it makes sense biologically. If two species are consistently distinguished by one or more diagnostic morphological differences than there is no gene flow between them. Lymantriids, referable to superfamily Noctuoidea, are commonly known as tussock moths having immense economic, environmental and aesthetic importance and are well represented in all Zoogeographic realms with 2500 species referable to 360 genera (Holloway, 1999). During the present studies, the different species of family Lymantriidae have been studied by taking into account the genitalia characteristics to facilitate the authentic identification and delimitation of species.

Keywords- Species, Ditrysian, Systematics, Genitalia.

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Original Research Article

Exploring the Scope of Panchakarma in Fever and Febrile Complications – A Critical Review

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Abstract

Introduction: The Ashtavaidya Tradition and traditional vaidya families in Kerala has added a lot to the unique flourishment of Panchakarma in Kerala. The time – tested clinical wisdom has added a lot to the arena of procedures through which effective and safe management is possible to many challenging health dilemmas of the present time like fever and febrile complications. Many of the procedures remained practiced to a particular region because the traditional books were written in regional language.

Aim: To explore the scope of research in Panchakarma in fever and febrile complications.

Review results: a). Sthanya (breast milk) therapy: Sthanya is particularly advised in fever and its associated complications through nasal route (nasya karma). In case of loss of consciousness associated with fever with less kapha nvolvement, sthanya mixed with mrdweeka, maricha, sunti, pippali, saindhava etc are used for nasya karma. Lohithanda (egg of red ants) which is a high source of protein and acetic acid, in sthanya is also advised for nasya karma in the same condition. Sthanya particularly contains casein, which possess considerable anti-infection property, may be absorbed through nasal mucosa in nano-particulate form along with drugs used very quickly through arachnoid matter sleeve extending along the olfactory nerve. Immuno- modulatory action of cytokines and lactoferrin in breast milk also contribute much during an infectious febrile condition when it is administered through nose. Also, k-casein, lactoferrin and lacto-peroxidase have considerable anti-microbial property which is of extreme importance in the perspective of communicable diseases. Secretory IgA, IgM,IgG in breast milk help to boost up the mechanism of immunity. b). Nadi sweda (Sudation using tubular instrument) with dasa pushpa: Leaves of five plants of dasapushpa boiled in dhanyamla is prescribed for nadi-sweda in febrile condition. This help to overcome the jadatha and sthabdhatha (tiredness and stiffness) caused by fever. c). Kulatha Pinda Sweda (sudation with potali made of horse gram), which is hot in its potency is advised in condition of fever associated with complications like heavyness.

Keywords- Panchakarma, Fever, Febrile complications.

Comparison of Antioxidant Activity of in vivo and in vitro Leaf Explants of Piper longum

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Abstract

Piper longum is a medicinal plant which has antioxidant property. Antioxidant property of plants can scavenge free radicals and protect the cell from oxidation. It helps to cure myocardial ischemic disease, a serious cardiac problem. Hot methanolic extract from leaf explants of plant was prepared for *in vivo* and *in vitro* studies and its antioxidant activity was determined by estimating Total phenolic content (TPC) and 2,2- diphenyl 1-2-picryl hydrazyl (DPPH) radical scavenging activity. As compounds responsible for the antioxidant effects are phenolic, hence, a preliminary assessment was done with total phenolic assay. Oxidative stress which can be relieved by antioxidant, are caused mainly by free radicals. Thus, it is important to measure free radical scavenging activity using DPPH. Comparison of *in vivo* and *in vitro* results showed the effect of tissue culture on extent of antioxidant activity of plant which is informative in terms of medicinal value of the plant. Further, the percentage of TPC and percentage of DPPH radical scavenging capacity were evaluated.

Objective: To estimate antioxidant activity of leaf extract of the medicinal plant *Piper longum* by two methods namely, TPC and DPPH radical scavenging capacity. Further there was comparison of *in vivo* and *in vitro* activity of hot methanolic leaf extract by calculating percentage of TPC and percentage of DPPH from optical densities.

Material and methods: *Piper longum* plant was procured from botanical garden of National Research Institute of Basic Ayurvedic Sciences, Nehru Garden, Pune, Maharashtra, India.Chemicals and reagents used were Gallic acid, sodium carbonate, 2, 2 – diphenyl 1-2-picryl hydrazy1 (DPPH), Folin-Ciocalteu reagent, concentrated sulphuric acid.

Preparation of Hot Extracts: 10 gm leaf powder of *Piper longum* was taken and mixed with 100 ml methanol it was then heated at 50°C and kept on shaker overnight, next day it was dried in rotavapour, then filtered using whatman filter paper and was preserved at 4°C.

For in vivo analysis: For TPC: Experiment was carried out using 9 test tubes .1st test tube was kept as blank. Gallic acid solution was pipetted in following order 0.1 ml, 0.2 ml, 0.3 ml , 0.4ml, 0.5 ml in 2^{nd} , 3^{rd} , 4^{th} ,5^{th} and 6^{th} test tube , and 1 ml each in 7^{th},8^{th} and 9^{th} test tube respectively. Plant sample was pipetted in following order in 200 µg/ml, 400 µg/ml and , 800 µg/ml in 7^{th} 8^{th} and 9^{th} test tubes respectively . Then DW was pipetted in following order in 2.5 ml,2.4 ml, 2.3 ml, 2.2 ml, 2.1 ml, 2 ml in 1^{st}, 2^{nd}, 3^{rd}, 4^{th} ,5^{th} and 6^{th} test tube , and 1.5 ml each in 7^{th} 8^{th} and 9^{th} test tube respectively, , further 0.5 ml of folin reagent was added to each tube and were kept for 3 min at room temperature there after 1 ml of 20% sodium carbonate was added and incubated at room temperature for 90 min and absorbance of blue colour developed was read at 760 nm using spectrophotometer [1].

For DPPH: Experiment was carried using 6 test tubes (sterile) .1^{stt} tube was marked as blank, methanolic extract of plant leaf was pipetted in following order 1 ml, 2 ml, 3 ml, 4 ml,5 ml in 2^{nd} ,3^{rd} ,4^{th} ,5^{th} and 6^{th} test tube respectively. Methanol was added in following order 5 ml, 4 ml, 3 ml, 2 ml, 1 ml in 1^{st} ,2^{nd} ,3^{rd} ,4^{th} ,5^{th} test tubes respectively. No methanol was added to the last tube, then DPPH quantity 5 ml was added to each test tube and was kept for 20 min at a temperature of 27°C. Using methanol as blank OD of sample was measured at 517 nm [1].

For in vitro analysis: Callus was grown by tissue culture method using three hormonal combination i.e, Indole acetic acid (IAA), Benzyl amino purine (BAP) and kinetin (KIN) to carry *in vitro* study. In vitro grown callus were cut into pieces and crushed with the help of motor and pestle with methanol and then 10 ml of solution was added to conical flask containing 90 ml of water. The steps for preparation of hot extract and protocol for detection of antioxidant activity (TPC and DPPH radical scavenging capacity) were same as *in vivo*.

Results: In the case of TPC, it was 43.75% for *in vivo* and 44.75% for *in vitro* leaf extract whereas in the case of DPPH, it was 1.60% for *in vivo* and 5.10% for *in vitro* leaf extract. It was concluded from the calculations that *in vitro* results were better than *in vivo* results.

In case of TPC positive control was gallic acid. The percentage TPC for gallic acid was 40.69% for *in vivo* case for leaf. The percentage TPC for gallic acid was 51.32% for *in vitro* for leaf. Percentage TPC for test sample *in vivo* was 43.75% for leaf and for *in vitro* case it was 44.75% for leaf.

In the case of DPPH positive control was considered in which there was no methanol but only sample. The Percentage of positive control in case of *in vivo* was 1.60% for leaf and in case of *in vitro* it was 5.10% for leaf.

Discussion: Antioxidant property was determined by estimation of Total Phenolic Content (TPC) [2]. In the present work, control sample in case of TPC was gallic acid and for DPPH radical scavenging capacity last test tube was considered as control in which there was no methanol. In present work there was dose dependent increase in percentage TPC for hot methanolic leaf extract.Optical densities evaluated in ELISA

reader plate are shown in table 1. In previous studies which was carried on fruit extract there was also increase in percentage TPC [1]. Antioxidant property was also determined on the basis of the ability of DPPH to scavenge free radicals [3]. In present work, at low concentration of leaf extract, percentage DPPH radical scavenging capacity is high and vice versa. Optical densities evaluated in ELISA reader plate are shown in table 2.Comparitive analysis between percentage TPC and percentage DPPH *in vivo* and *in vitro* shows that *in vitro* results were better than *in vivo* results , but results were different in case of previous studies carried on fruit extract of *Piper longum,* in which there was dose dependent increase in percentage DPPH radical scavenging capacity [4,5].

Conclusion: *In vitro* results were better as compared to *in vivo* results for leaf explants in case of TPC and DPPH radical scavenging capacity for determination of anti oxidant activity which is informative in terms of medicinal value of the plant that can be proved beneficial in treatment of diseases related to oxidative stress.

Key Words- *Piper longum*, TPC, DPPH, antioxidant activity, methanol.

1. Introduction

Antioxidant are inhibitors of oxidation, which prevents the oxidation and protect the cell from damage. Plant possessing antioxidant activity are of great use in treatment of various human related ailments. Oxidative stress results due to oxidants or free radicals and this stress is major cause of many diseases. It can cause damage that can result in cancer, ischemia, aging, rheumatoid arthritis, etc. Antioxidant property of *Piper longum* was determined by performing two methods TPC and DPPH radical scavenging capacity. Hot methanolic leaf extract of the explants was prepared and its antioxidant activity was evaluated. Comparison of *in vivo* and *in vitro* leaf explants yields different results which further provide information about medicinal value of the plant in each case.

2. Equations

$$\text{Percentage TPC} = \frac{\text{Actual Concentration}}{\text{Observed Concentration}} \text{X } 100 \qquad (1)$$

$$\text{Percentage DPPH} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \text{ X } 100 \qquad (2)$$

3. Tables

Table 1: Antioxidant property for TPC

Tube number	OD (*in vivo*)	OD (*in vitro*)
1.(Blank)	0.7	0.64
2.(0.1ml gallic acid)	1.25	1.25
3.(0.2ml gallic acid)	1.72	1.68
4.(0.3ml gallic acid)	1.88	1.81
5.(0.4ml gallic acid)	1.94	1.97
6.(0.5ml gallic acid)	1.98	1.93

7.(200µg/ml plant sample+1ml gallic acid)	1.85	1.69
8.(400µg/ml plant sample+1ml gallic acid)	1.65	1.53
9.(800µg/ml plant sample+1ml gallic acid)	1.60	1.48

Table 2: Antioxidant property for DPPH radical scavenging capacity

Tube number	**OD (*in vivo*)**	**OD (*in vitro*)**
1.(Blank) (5ml methanol+5ml DPPH)	1.25	0.64
2. 1ml methanolic leaf extract +4ml methanol+5ml DPPH	1.12	1.25
3. 2ml methanolic leaf extract +3ml methanol+5ml DPPH	1.13	1.68
4. 3ml methanolic leaf extract 2ml methanol+5ml DPPH	1.20	1.81
5. 4ml methanolic leaf extract +1ml methanol+5ml DPPH	1.21	1.97
6. 5ml methanolic leaf extract +0ml methanol+5ml DPPH	1.23	1.93

4. Conclusion

In vitro results were better as compared to in vivo results for leaf explants in case of TPC and DPPH radical scavenging capacity for determination of anti oxidant activity which is informative in terms of medicinal value of the plant that can be proved beneficial in treatment of diseases related to oxidative stress.

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Comparative Analysis on Effect of Antibacterial Activity of Piper longum leaf Explants in vivo on Escherichia coli and Bacillus subtilis

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Abstract

Antibacterial activity of *Piper longum* can protect from bacterial infection. *Piper longum* prevents stomachache, Diseases of spleen, respiratory tract, etc.Study on bacteria *Escherichia coli* and *Bacillus subtilis* gives information about the extent of inhibitory effect of leaf explants of the plant. There was comparison of effects in terms of percentage inhibition by preparing hot ethyl acetate extract of leaf explants, which was further calculated for both the bacterium.

Objective: To evaluate antibacterial activity of leaf explants of *Piper longum* on gram negative and gram positive bacteria i.e, *Escherichia coli* and *Bacillus subtilis* respectively by preparing hot ethyl acetate extract of the leaf explants and to evaluate the inhibitory effects by calculating percentage inhibition in each case.

Material and methods: *Piper longum* plant was procured from botanical garden of National Research Institute of Basic Ayurvedic Sciences, Nehru Garden, Pune, Maharashtra, India**.** Bacterial culture i.e, of *Escherichia coli* and *Bacillus subtilis* was procured from laboratory of fergusson college,Pune,Maharashtra. Chemicals used were Dimethyl Sulphoxide (DMSO) and Ampicillin.

Nutrient broth preparation: A media composition of Tryptone - 10 gm/l, Sodium chloride - 10 gm/l, Yeast extract- 5 gm/l and Distilled water – 1000 ml was prepared and adjusted to a pH of 7.2. Sample Dilutions: Preparation of Dimethyl Sulphoxide (DMSO) solution: (10 ml)-1ml DMSO was added to 9 ml sterile nutrient broth in sterile tube. 10 mg leaf extract was added to 1ml DMSO stock solution.

Preparation of Hot Extracts

10 gm leaf explant powder of Piper longum was taken and mixed with 100 ml ethyl acetate it was then heated at 50°C and kept on shaker overnight, next day it was dried in rotavapour, then filtered using whatman filter paper and was preserved at 4°C.

Serial dilutions: For leaf extract dilution was done in the ratio 1:1(0.5 ml from stock solution was added to 0.5 ml DMSO), 1:2(0.5 ml from stock solution was added to 1ml DMSO), 1:4 (0.5 ml from stock solution was added to 2 ml DMSO). These Dilutions were prepared for hot extract of leaf.

Preparation of Bacterial cultures: 100 ml of nutrient broth was prepared in 2 conical flask, each were autoclaved and then loopful of bacterial culture of *Escherichia coli* and *Bacillus subtillis* was inoculated in two flasks. It was then incubated on shaker for 2 hrs (O.D was 0.3 at 620 nm).Inhibition studies was done on ELISA plate.

Hot extract of leaf and cell suspension of *Escherichia coli* and *Bacillus subtillis*: 100 µl of cell suspension and 100 µl of each dilutions (hot extract of leaf) was pipetted in triplicate in 1st row of plate., In case of positive control 100 µl of cell suspension was added to 100 µl of ampicillin solution (1 ml sterile distilled water was mixed with10 mg antibiotic and stored at 4°C) in 2nd row of plate it was pipetted in each well . In case of solvent control 100µl of DMSO was added to 100 µl of cell suspension in 3rd row of plate,. In case of negative control 200 µl of cell suspension was added in each well in 4th row,. The Plate was incubated at 37°C for 48 hrs. Absorbance was taken at 620 nm on ELISA reader & extent of inhibition was calculated [1].

Results: In the case of *Escherichia coli*, for 1:1 dilution of the plant, hot extract of leaf, The percentage inhibition was 4.32%, for 1:2 dilution it was 3.69%, for 1:4 dilution it was 2.25%. All the dilution was taken in triplicates. On the other hand in the case of *Bacillus subtillis,* For 1:1 dilution of the plant hot extract of leaf the percentage inhibition was 5.18%, for 1:2 dilution it was 3.17% for 1:4 dilution it was 2.17%. All dilutions were taken in triplicates in ELISA reader plate. Further, The results of positive control (ampicilin) for *Escherichia coli* and hot extract of leaf explants used as test sample was 74.47% whereas for *Bacillus subtillis* the percentage inhibition for control was 71.23%.

Discussion: In the present work, in case of antibacterial activity there was dose dependent increase in percentage inhibition, Ampicillin was taken as positive control, this antibiotic has antibacterial activity for bacteria used. It is used against *Piper longum* as control to check the extent of antibacterial activity of its leaf explant.Previous studies which was carried on fruits of *Piper longum* to check its antibacterial activity also showed dose dependent increase in the percentage of inhibition [2, 3, 4].The results of *Escherichia coli* and *Bacillus subtilis* showed less percentage of inhibition then their respective positive control.Moreover the result of percentage inhibition for *Escherichia coli* was better than that of *Bacillus subtilis.*The optical densities for *Escherichia coli* and *Bacillus subtilis* are shown in table 1 and table 2 respectively.

Conclusion: Results give information about the plant's medicinal value and its activity against two bacteria namely *Escherichia coli* and *Bacillus subtillis.* Comparative analysis between these two bacteria showed that leaf explants of *Piper longum* has better inhibitory effect on
Escherichia coli as compared to *Bacillus subtillis.* So, it is necessary to carry study on the above bacterium to gain knowledge which is used to cure diseases caused by bacterial infection.

Key Words- *Piper longum*, antibacterial activity, *Escherichia coli, Bacillus subtilis*

Micro-organisms such as bacteria are causative agents of many diseases.Diseases caused by bacterial infection can be cured if antibacterial agents are used against them. Inhibitory effect of antibacterial property of leaf explants of *Piper longum* are beneficial to gather information necessary for treatment of diseases. Two bacteria studied in the present case are *Escherichia coli* and *Bacillus subtilis. Escherichia coli* is gram negative bacteria and is causative agent of the diseases like cholecystytis, bacteremia, cholangitis, diarrhea, etc. *Bacillus subtilis* is gram positive bacteria and it is non-pathogenic, it does not causes disease. In the present work, there is comparison of inhibitory effect of *Piper longum* leaf explants on gram positive and gram negative bacteria. The percentage inhibition in each case was determined. Further, results of inhibition were better in *Escherichia coli* as compared to *Bacillus subtilis*

2. Equations

$$\text{Percentage of Inhibition} = 100 - \left(\frac{\text{Absorbance of test sample}}{\text{Absorbance of Control Sample}}\right) \text{X } 100 \quad (1)$$

$$\text{Percentage of Inhibition} = 100 - \frac{\text{Mean of O.D. of the triplicates}}{\text{Mean of control O.D.of corresponding triplicates}} \text{X } 100 \quad (2)$$

3. Table

Table 1: Antibacterial property (*in vivo*) for Hot extract of leaf and cell suspension of E.coli

	1	2	3	4	5	6	7	8	9
A E. coli+Plant sample	0.911 (1:1)	0.912 (1:1)	0.921 (1:1)	0.908 (1:2)	0.904 (1:2)	0.901 (1:2)	0.911 (1:4)	0.909 (1:4)	0.905 (1:4)
B E. coli+Ampicillin Positive control	0.239	0.240	0.241	0.242	0.243	0.245	0.241	0.245	0.239
C DMSO+ E.coli Solvent control	0.343	0.337	0.323	0.341	0.341	0.343	0.347	0.337	0.332
D E.coli Negative control	0.951	0.956	0.961	0.939	0.941	0.937	0.937	0.938	0.936

Table 2: Antibacterial property (*in vivo*) for Hot extract of leaf and cell suspension of B.subtilis

	1	2	3	4	5	6	7	8	9
A E. coli+Plant sample	0.912 (1:1)	0.867 (1:1)	0.567 (1:1)	0.956 (1:2)	0.808 (1:2)	0.678 (1:2)	0.909 (1:4)	0.832 (1:4)	0.689 (1:4)
B E. coli+Ampicillin Positive control	0.238	0.240	0.241	0.244	0.246	0.234	0.212	0.234	0.267
C DMSO+ E.coli Solvent control	0.341	0.343	0.345	0.340	0.341	0.356	0.311	0.321	0.346
D E.coli Negative control	0.897	0.698	0.961	0.939	0.941	0.937	0.937	0.938	0.936

4. Conclusion

Results give information about the plant's medicinal value and its activity against two bacteria namely *Escherichia coli* and *Bacillus subtillis*. Comparative analysis between these two bacteria showed that leaf explants has better inhibitory effect on *Escherichia coli* as compared to *Bacillus subtillis*. So, it is necessary to carry study on the above bacterium to gain knowledge which is used to cure diseases caused by bacterial infection.

.Acknowledgment

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IOCBS Online Biology Conference 2017

Original Research Article

Antipyretic Activity of Pterolobium hexapetalum (Roth.) Sant. and Wagh. Stem Bark Extracts

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Abstract

Pterolobium hexapetalum (*Caesalpiniaceae*) is one of the important herbal medicines against chest pain, fever, cough, tooth ache, dog bite, diarrhoea, ulcer, jaundice, skin disorders, constipation, piles and venereal diseases. Also possess high quantities of phytoconstituents in leaf, stem bark, flower and fruit extracts like flavonoides, alkaloids, phenols, glycosides, saponins steroids, tannins and quinines. *P. hexapetalum* extracts also proved as effective antimicrobial, antiulcerous, antidiarrhoel and antioxidant herbal drug through in – vitro and in – vivo studies. *P. hexapetalum* stem bark extracts have been evaluated for their antipyretic activity against yeast – induced pyrexia in rats. The methanol as well as water extracts of the stem bark showed potential antipyretic activity. It was observed that methanol extract at a dose of 400 mg/kg body weight significantly elevated body temperature of rabbit showed maximum antipyretic activity than water extract. The effect produced was comparable with the standard antipyretic drug *paracetamol*. Hence present investigation reveals the antipyretic activities of the methanolic and water extracts of the stem bark extracts of *P. hexapetalum.*

Key words: Antipyretic, Albino rats, *Pterolobium hexapetalum*, *Paracetamol*, Yeast

Introduction: Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection [1]. Fever is associated with symptoms of sickness behaviour which consist of lethargy, depression, anorexia, sleepiness & inability to concentrate. Antipyretic medication can be effective at lowering the temperature which may include the affected people's comfort [2]. Plants have been a major source for new drug design. Traditional use of medicinal plants with antipyretic activity is a common worldwide feature of many ethno botanical cultural systems. In ethno botany, plants with naturally occurring antipyretic activity are commonly referred as febrifuges [3 & 4].

Pterolobium hexapetalum (Roth.) Sant. and Wagh. ("Yerra checki") is an extensive, armed straggling spiny shrub and herbal medicine used by the chenchu tribes of Nallamalai forest region of Mahanandi hills. *P. hexapetalum* leaf and fruit paste is used to cure diarrhoea, constipation and piles [5 & 6]. Leaf, stem bark, flower and fruit extracts resulted high quantities of alkaloids, flavonoids, phenols, glycosides, tannins, quinones and steroids. And also proved as effective antifungal against *Aspergillus niger* and *Candida*

albicans at 10 mg/well, with MIC values 0.625 and 1.25 mg respectively. [7]. Also reported as effective antibacterial against four pathogenic bacterial strains with MIC values ranges from 0.312-1.25 mg [8]. Hence the *P. hexapetalum* stem bark methanol and aqueous extracts pyloric effects in yeast induced pyrexia albino wister rats at 200 and 400 mg/kg b.wt were tested to prove its efficacy more scientifically to that of the traditional herbal use.

Materials & Methods:

Objectives: The stem bark methanol & aqueous crude extracts has to subject for toxicity studies.

Antipyretic activity by yeast induced pyrexia was carried out.

Collection and identification of the plant material: The stem bark was collected from Nallamalla forest of Mahanandi, Kurnool, AP. The taxonomic identification of the plant was confirmed by Prof. N. Yasodamma, Department of Botany, Sri Venkateshwara University, Tirupathi, and Andhra Pradesh, India.

Preparation of the crude extracts: Fresh stem bark was washed shade dried, powdered and 70 g each were soaked and extracted with water after 72 hrs the filtrate was dried on water bath. The dried powders each 40 g were extracted in a soxhlet apparatus using 200 ml of solvent methanol. The filtrates were concentrated on rotavapour and dried. All extracts were stored at 40C in refrigerator until further use.

Animal selection: Wister albino rats of both sexes of either weighing about 150 – 200 g were employed for this study. The animals were acclimatized to standard laboratory conditions (temperature 25±2oC) and maintained on 12 hours light; and 12 hours dark cycle. They were fed with ad libitum. The experimental protocol was approved by institutional animal ethical committee in the Resolution No.12/2011-2012/ (i) 438/01a/CPCSEA/IAEC/SVU/NY-BK/dt: 19/11/2011.

Acute toxicity Study: It was carried out as per the 423 guidelines set by OEC (Organisation for economic co-operation and development). Albino rats (n=10) of either sex selected by random sampling technique were used for the study. The aqueous and methanol extracts were administered at the dose levels of 500, 1000, 1500, 2000, 2500, 3000 and 3500 mg/kg body weight by oral gavage and observed for 14 days.

Antipyretic test: Yeast induced pyrexia method. The albino rats were randomly distributed in control and test groups of six animals each. They were fed with standard laboratory diet *ad libitum* and allowed free access to drinking water [9]. The animals were kept in 12/12 hours dark-light cycle. Fever was induced in rats by subcutaneous injection of 20% w/v of brewer's yeast suspension (10 ml/kg) in to animal's dorsum region. 19 h after yeast injection, the rectal temperature of each rat was measured using a thermometer. Only rats that showed an increase in temperature of at least 0.7 ^{o}C were employed for the experiments. The methanol and water extracts (200 and 400 mg/kg) or 10% v/v propylene glycol solution (10 ml/kg) was administered orally and the temperature was measured at 0, 1, 2 and 3 h after drug administration.

Statistical significance: All the data are expressed as mean ± SEM. The values obtained for the above parameters with the extracts were compared with control group using one way ANOVA followed by

Dunnett's test. The values of $p<0.05$ and $p<0.01$ were considered to indicate a significant difference between the groups.

Results:

Acute toxicity study (LD50): Stem bark aqueous and methanol extracts were studied for acute toxicity at different doses of 500, 1000, 1500, 2000, 2500, 3000 and 3500 mg/kg b.wt. and observed for 14 days. The extracts found devoid of mortality of the animals in addition, no toxic symptoms were observed also food and water intake was not affected during the study period. So these extracts did not show any significant toxicity on Wister albino rats. Hence 3500 mg/kg was considered as LD50 cut off value. So the doses selected for experiment as per OECD guidelines 423 and fixed up to a maximum of 140 mg/kg (1/25th of 3500 mg/kg).

Antipyretic activity: *Paracetamol* is a common antipyretic agent, which is safe in therapeutic doses and analgesic compound available for many years for oral administration since intravenous infusion was shampered by water insolubility. Experimental results exhibited that both the extracts at a dose of 400 mg/kg body weight showed maximum activity and to maintain a normal body temperature and reduce yeast induced elevated rectal temp in rat and their effect are comparable to that of standard antipyretic drug. The results indicated that highest antipyretic activity of methanol extract when compared to that aqueous extract.

Table: 1: Antipyretic effect of *Pterolobium hexapetalum* stem bark extracts in albino rats

Treatment /Dose (mg/kg)	**Initial Temperature (^{0}C)**	**Average Rectal Temperature ^{o}C in hour ± SEM**			
		0 Hour	**1 Hour**	**2 Hour**	**3 Hour**
Control	37.50 ± 0.25	37.60 ± 0.16	37.70 ± 0.12	37.90 ± 0.14	38.10 ± 0.24
***Paracetemol* - 150mg**	37.48 ±0.20	39.10 ± 0.24	38.60 ± 0.66	38.48 ± 0.40	38.0 ± 0.46**
Methanol -200mg	37.20 ± 0.24	37.54 ± 0.92**	37.40 ± 0.70*	37.22 ± 0.48	37.20 ± 1.77
Methanol -400mg	36.20 ± 0.05	37.37 ± 0.29	36.40 ± 1.95	36.20 ±1.77	37.52 ± 0.70*
Aqueous -200mg	37.40 ± 0.40	37.77 ±1.46	37.42 ± 0.70*	37.00 ± 0.25	37.63 ± 0.76
Aqueous -400mg	36.10 ± 0.05	37.20 ± 0.66	36.40 ± 1.95	36.22 ±1.77	36.90 ±0.14

All the data are expressed as mean ± SEM, n=6, * $p<0.05$ and ** $p<0.01$ when compared with control group One way ANOVA followed by Dunnett's test.

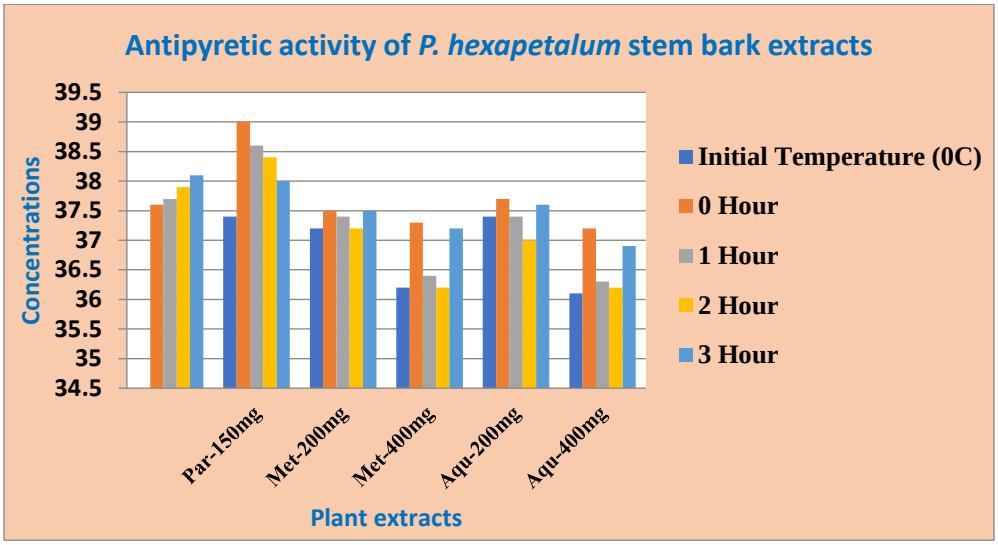

Figure: 1

Discussion:

The acute toxicity study, antipyretic properties of *P. hexapetalum* methanol and water extracts were investigated in the present study. It was found to be safe and no mortality was observed to a dose as high as 3500 mg/kg. The acute toxicity result reveals that this plant might be considered as a broad non – toxic one. Now day's traditional plants are the main sources for isolation of potent drugs. It was found that the stem bark extracts of *P. hexapetalum* having the antipyretic effect. It reveals that methanol extract at a dose of 400 mg/kg body weight showed maximum antipyretic activity. It maintaining normal body temperature and reducing boiled milk induced elevated rectal temperature in rats and their effect are comparable to that of standard antipyretic drug *paracetamal*. Antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis [10]. The antipyretic activity may be due to the presence of phytochemicals such as saponins, flavonoids, glycosides, alkaloids and anthraquinones have been reported to exhibited acute and antipyretic activity in rats [11, 12 &13]. The present study therefore supports the claims of traditional medicine practitioners as an antipyretic remedy.

In conclusion, this study provides evidences for the antipyretic activity of *P. hexapetalum* which could partly contribute to its ethno medical use. However, further investigation is required to isolate the bioactive constituents responsible for these activities and to elucidate the exact mechanisms of action.

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Original Research Article

Acute Dacryocystitis (Pooyalasa in Ayurveda) – A Case Report

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Abstract

Inflammation of lacrimal sac is called dacryocystitis. It is usually secondary to obstruction of nasolacrimal duct and is commonly caused by streptococcus or staphylococcus. Acquired dacryocystitis may be acute or chronic. Acute dacryocystitis may present as a lacrimal abscess which may then burst spontaneously and if not intervened in time can result in a permanent lacrimal fistula, orbital cellulitis or cavernous sinus thrombosis. Dacryocystorhinotomy is usually done after the active infection is controlled. The disease *pooyalasa* described in Ayurveda has features similar to that of acute dacryocystitis. The description of the disease in *Ashtanga hridaya* explains two stages. The stage of inflammatory swelling or *vrana sopha* which may burst own its own to give rise to the stage of a *vrana* or ulcer. *Pooyalasa* is a *pilla roga* which can developing into a chronic recurrent pathology is not properly treated. A 31 year old male patient, a diagnosed case of Steven Johnson syndrome for the past 15 years developed sudden onset severe pain, swelling and redness of left eyelids and upper part of cheek and fever, with positive regurgitation test. A clinical diagnosis of acute dacryocystitis or *pooyalasa* was made and he was treated along the lines of *vranasopha chikitsa* with *Panchathiktakam kashayam* 90 ml tds, *Amrutharishtm* 30 ml tds, T.*Sudarsanam* 2 tds, T.*kaisoraguggulu* 2 tds, *Vidalaka* with *mukkadi gulika* and *lepana* over the swelling with *krishnadi gulika tds*, *gandoosha* with *triphala kwatha* and *jalookavacharan*a around the swelling. On bursting of abscess, with the aim of *vrana sodhana* and *ropana,* mild *peedana* was done around the ulcer and *jatyadi ghrita* was applied over the ulcer to promote pus drainage, *patoladi ghritha* was given 10g bd internally. After 1 week *durvadi ghritha* was applied over the ulcer to promote healing and once the ulcer, healed *agnikarm*a was done to prevent recurrence. Ulcer healed completely in one month. This case demonstrates the ability of Ayurvedic management protocols to effectively manage acute presentations. Research needs to be carried out to develop standardized management algorithms to deal with specific acute diseases.

Keywords: Dacryocystitis, *Pooyalasa, Ayurveda,* Management

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Original Research Article

Role of Genitalia in family Brahmaeidae (Lepidoptera: Bombycoidea)

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Abstract

Insects are the most cosmopolitan, polyphagous and varied group of living organisms on our planet earth (Prado and Zucchi, 2012). The order Lepidoptera is one of the most dominant order in class Insecta, having 1,57,424 species referable to 15,578 genera comprising of moths and butterflies (Zhang, 2013). The family Brahmaeidae of superfamily Bombycoidea is a small but spectacular family found in the African, Oriental and Palaearctic regions. In this family, only four genera can be recognised i.e., Oriental and Palaearctic genus *Brahmaea* Walker; European genus *Acanthobrahmaea* Sauter; a monotypic Chinese genus *Calliprogonos* Mell and an Afrotropical genus *Dactyloceras* Mell. In 1986, Sauter placed two genera i.e *Calliprogonos* Mell and *Dactyloceras* Mell in a separate subfamily, Dactyloceratinae, but this division is probably superfluous in such a small family. The genus Brahmaea Walker is represented by only two species namely *Brahmaea wallichii* Gray and *Brahmaea hearseyi* White. The significance of the morphological details of the external genitalia in resolving the taxonomic identities are well recognized in the insects in general and particularly in order Lepidoptera (Miller, 1968). During present studies, both these species i.e., *Brahmaea wallichii* Gray and *Brahmaea hearseyi* White have been collected from different localities of Himachal Pradesh. It is very difficult to differentiate these species on the basis of external morphological charcters. The male genitalic features such as uncus, gnathos, juxta, valvae and aedeagus of these species have been explored in detail and it has been found that these genitalic features proved to be the most reliable and important taxonomic tool in differentiating these closely related species. Hence the present study signifies the role of external genitalic features in differentiation and diagnosis of different taxa in family Brahmaeidae.

Keywords: Lepidoptera, Bombycoidea, Brahmaeidae, external genitalia.

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Original Research Article

MED-PDB: An Online Database of Medicinal Plants

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Abstract

Medicinal plants have been anticipated to be one of the most valuable resources in therapeutic practices for human diseases. A range of plants in the form of herbal medications have been publicized to be therapeutically significant in a large number of diseases, counting cancers, diabetes, autoimmune disorders, epidermal infections, dermatological disorders, etc. WHO also claims that medicinal plants are extremely important for the population of developing countries.

Plant extracts have been used in raw, crude as well as processed form. Despite the clear evidence of the medicinal usage of plants, there is no such central repository that houses all the medicinal plants and their usage. To fill this void, the present study aims to compile and curate the medicinal plants with their medicinal values from the published literature.

The present database host information on 147 plants species, 53 plant families & subfamilies, 435 types of diseases, 369 types of active compound and covers worldwide geographical distribution.

The database has been made freely available online at the URL http://genomeinformatics.dtu.ac.in/medicinalplant/.

We believe that the present database may help in pharmacological and clinical exploration of plant species revealing the subsequent role of active compounds in various human diseases.

Keywords-Medicinal Plants, Active Compounds, Disease, Database

1. INTRODUCTION

Several research model revolutions including both experimental and theoretical have made it plausible to fathom the modes of functioning of biological processes at molecular level. Computational paradigms of systems with extensive properties have also been proved to be the foundation for prognosis of biological behavior, giving rise to new discoveries and investigations [1]. In order to improve the efficiency of such biological transformation it is necessary to aid the processing, amalgamation, elucidation of the immense heap of biological data provided by various research communities. For many years, Databases have been

proved to be a usual way of dealing and handling vast oodles of information in miscellaneous fields, including industry, academic restraints, and government subdivisions.

The practice of database technologies has garnered the attention of a division of the biological community, but its operation has been sparse to a significant part of the community though these assets are followed by numerous people of the research community. This can circumscribe not only the usages of these data to its utmost volume but also guide to misuse of the data. Adding to it, many experimental biologists are figuring data on a massive scale and are in need of establishing and organizing their own databases.

The motivation of establishing a consolidated database is to explicate the process by which major database resources pertinent for plant research obtain, analyze, and beget their data accessible, to identify the current constraints and the future endeavors of these resources to promote application of databases to research problems and respective goals.

The notion of plant specific databases is matter to adjustment as researchers are flaring their room of research. Consider the availability of various gene sequences of many organisms from various data sources that has enabled clinicians and researchers to easily and rapidly access and compare the sequences of interest [2]. Adding to biological database based transformation, various single species databases are available which only deals with taxonomically related species for e.g. Databases for grains, cereals, and night shades. Other examples of unrestricted databases include the ones which are based on particular domains of data like metabolism, genome annotation, orthologous relationships etc.

Considering the storage and ongoing availability of such huge information there is a need to develop fairly independent database with good quality documentation and proper design to facilitate barrier free data exchange. A problem in building a database is the paucity of acknowledgment of this work as a real scientific effort. Many of the databases are public endeavors constructed with the help of software developers under the guidance of a biologist, sharing their experience via conferences may help in improving the problem. Widely held database based papers describe only the content and offer slight material on the design and operation of the software with no schema available.

Another crucial difficulty in this field is the inadequate capability to access and practically assimilate data from these multiple databases in an allied manner. Several types of databases along with plenty of software applications make it difficult for the researcher to extract the exact information in time and the representation of data in all the accessible form through these data sources puts an added load on researchers who wants to utilize the resource information.

Currently, we wish to deploy computer-readable data model of plant metabolomics based database MED-PDB, which incorporates a database schema for the formation of globally available plant metabolite information relating the active component to the effective metabolic target involved in various diseases, and a user interface to browse the certain defined attributes in context of disease. It will provide an enhanced

and automatous understanding about ongoing biological research transition and will help in better implementation of novel methods and technologies [6], [7].

2. Dataset and Feature Selection

For robust data prognostication, it is advantageous to incorporate several biological data sources and it has been realized that both plants and their medicinal uses are substantial for analyzing whether unknown active compound is used in a disease or not [3], [4], [5]. Consequently, for our model constructing motive we incorporated disease, botanical name, family, common name, Geographical Distribution, morphology of plant, type of extract, active compound, administration, biological target and PMID [1]. Medicinal uses of all the plants were the findings from NCBI. The parameters of Medicinal use of plants were defined according to type of extract and their correlation with disease cure etc.

3. Methodology

The primary data in the MED-PDB depicts affiliation of various plant extracts along with their active compounds to various diseases. All the complicated medical conditions that are presumed to be rife have been deliberated for the purpose of this work. The information on the relationship of plant active compound with disease was obtained from the articles published in subsections of NCBI.

The plant and disease associations for MED-PDB were manually curated from the germane articles published in PubMed extracted deploying the keywords such as “Disease AND Plant Metabolites” or “Disease name AND Active Compounds of plants”. The information from the current databases was sensibly evaluated and amended with apropos to the original articles. Then we created a data model deploying the free open-source version of the MySQL Workbench to minimize tautology [10]. Each ingress in MED-PDB contains the information on Disease, Botanical name, Family and Common Name, Geographical Distribution, Morphology of Plant, Type of Extract, Active Compound, Administration, Biological Target and PubMed ID (PMID).

4. User Interface

MED-PDB provides a comprehensible interface to query complete information on disease and plant association. Users can query the database through disease, botanical name, common name or family [3]. Further, MED-PDB interface permits the selection of the attributes, such that only the anticipated information on Plant-Disease association can be observed without encumbering the screen with data of least significance for the user [9].

5. Structure of Med-PDB Database

The MED-PDB database holds the data in the form of differentially categorized flat file format sub-locations. The user can access it through computer or mobile phone via the internet. The simple design of the database includes a front end search window and a back end database repository file.

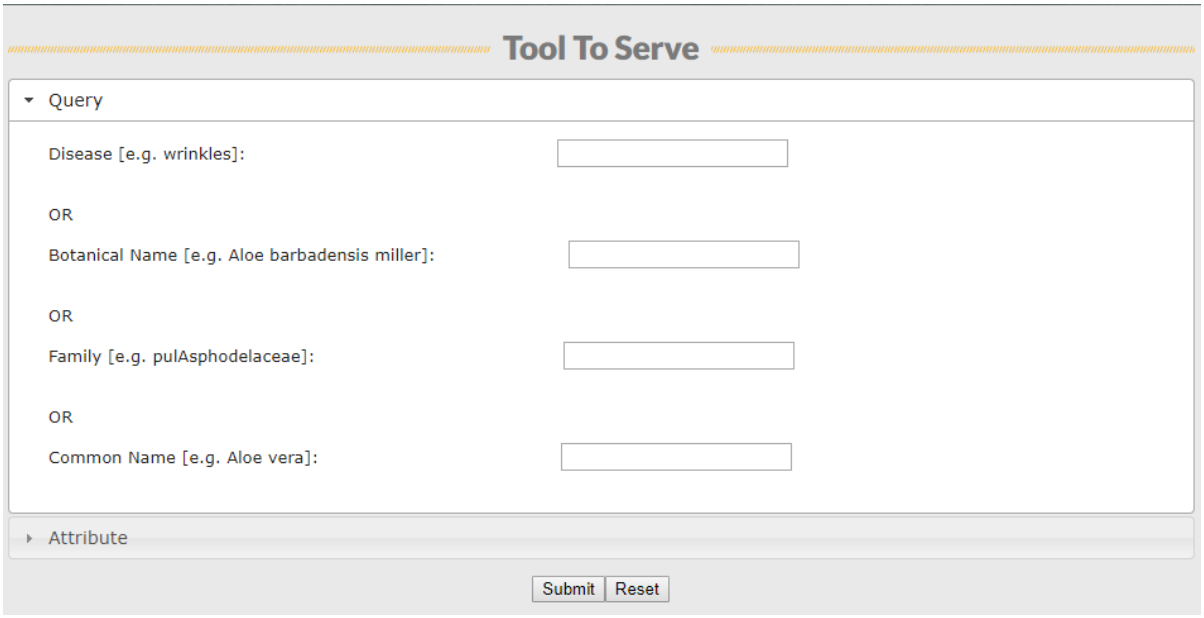

Fig. 1. The front end search window having four basic search section comprising Disease, Botanical name, Family and Common name that allow the user to make an input of interest.

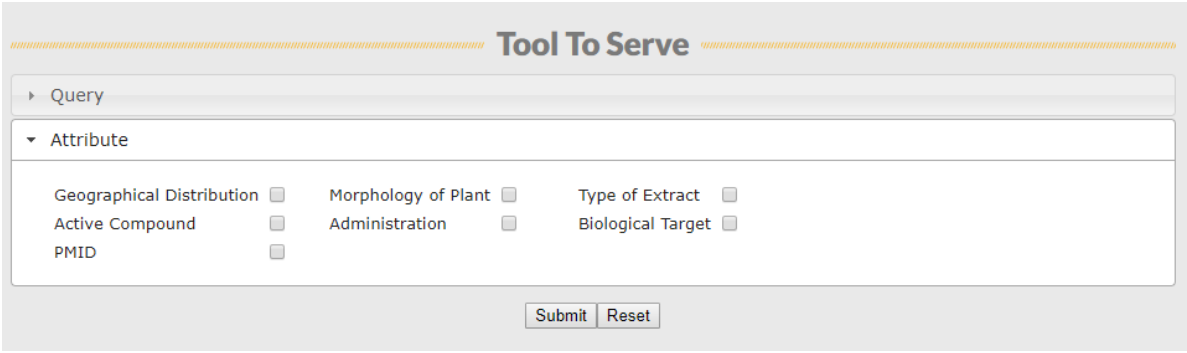

Fig. 2. A window which allows the user to select certain attributes in context of the search query

These attributes include Geographical Distribution, Morphology of Plant, Type of Extract, Active Compound, Administration, Biological Targets and PubMed ID (PMID) [4], [5].

	A	B	C	D	E	F	G	H	I	J	K
1	disease	BotanicalNam	Family	CommonNa	GeographicalI	MorphologyofPlan	TYPE OF EXTRA	ActiveCompound	Administration	BiologicalTarget	PMID
2	Burns	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	inner leaf gel	mannose-6-phosphate	Clear mucilaginous gel (pure a	collagen	19882025
3	Sunburn (and damage a	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	inner leaf gel	n/a	Clear mucilaginous gel (pure a	immunosuppressive	19882025
4	Analgesic	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	inner leaf gel	C-glucosyl chromone	Aloe vera leaf gel is used.	cyclooxygenase pat	8778246
5	Gingivitis	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	gel	Acemannan	Aloe vera leaf gel in mouthwa		23559789
6	Periodontitis	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	gel	n/a	Aloe vera leaf gel in mouthwa		25478478
7	Oral Cavity	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	gel	anthraquinones	Aloe vera tooth gel		25478478
8	Herpes simplex and Her	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	gel	anthraquinone aloin	Hydrophilic cream containing	0.5% aloe gel 3 time	19882025
9	Metastatic tumours	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	gel	glycoproteins (lectins)	The concomitant oral adminis	phorbol myristic ac	16819181;
10	Anti-Microbial	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	gel or juice	Lupeol, salicylic acid, a	A 100% Aloe vera juice obtained from the cold pre		17994359
11	Seborrheic Dermatitus	Aloe barbadensi	Asphodelaceae	Aloe vera	dry regions of Afr	succulent plant, fleshy	gel	n/a	30% aloe vera in a hydrophilic	n/a	19882025

Fig. 3. Back end flat data file with predefined sections containing the related information regarding the query.

6. Description of Attributes

1. **Disease: -** An abnormal condition with specific sign and symptoms. In our database we have provided information about 435 types of medical conditions.
2. **Botanical name: -**International code for nomenclature of distinct plant species. It defines the species and genus information. We have provided information about 147 plant species collected from the manually peered data.
3. **Family:** - It defines the homologous sharing group which contains various plant species of different genera's. MED-PDB holds description about 53 plant families and subfamilies.
4. **Common Name:** - A common epithet of a plant. It is very useful when a researcher wants to get the information about the role of a traditional plant in any disease.
5. **Geographical Location:** - Denizen of the medicinal plant. MED-PDB covers the world-wide distribution of various plants.
6. **Morphology of Plant:** - MED-PDB provides the information about the external appearance of the plant.
7. **Type of Extract:** - For medicinal purposes plant extracts are prepared in various different ways by taking different plant parts like root, stem, leaf etc. MED-PDB also contains the information about the 369 type of extracts used for the experimental purpose.
8. **Administration:** - During clinical procedure the way used to administer the drug determines a crucial role [4]. MED-PDB search output is able to give the information to the user about the same.
9. **Active Compounds:** - Use of various plant parts for the medicinal purpose depends on the type of target being used. It is necessary to know the nature of active compound in order to determine the proper

functioning or interaction of target with the target compound. Plants are said to be the abundant source of various medically important Active compounds for e.g. Alkaloids, Terpenoids, Glycosides, Natural Phenol, Phenazines, Biphenyls etc. MED-PDB provides information about the type of active compound involved in disease metabolism.

10. **Biological Target:** - Functioning of any target compound depends on the nature of target it is going to interact with. MED-PDB provides the information about the Biologically Active site interacting with active compound.
11. **PMID:** - It is the unique identification number assigned for each and every record which is stored in PubMed. The section of MED-PDB can provide the information about the literature for the whole work in just one click.

7. Web Implementation

For the expedition of data retrieval, user-friendly web interface was evolved. XHTML and CSS were deployed for forming presentation layer of MED-PDB, the application server deployed was Apache. MySQL was used for backend database and PHP was used as a programming language [8].

8. Database Accessibility

The basic unit of MED-PDB is the various medicinal properties of plants, which is depicted in the online delivery model of database as a Variant Report. Synopsis is provided for maximum plant species, including the various disease, botanical name, family, common name, Geographical Distribution, morphology of plant, type of extract, active compound, administration, biological target and PMID. Synopsis is ensued by the list of PMID curetted for the variant. Each study divides into a set of observations, with each observation comprising five core fields of data.

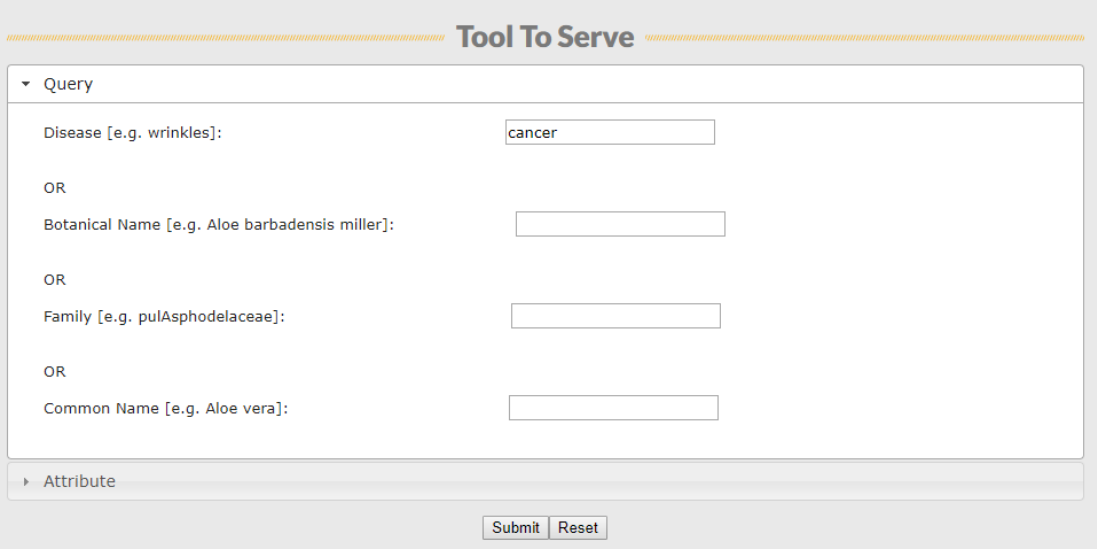

Fig. 4. Introducing Cancer as the query input in front end search window at MED-PDB.

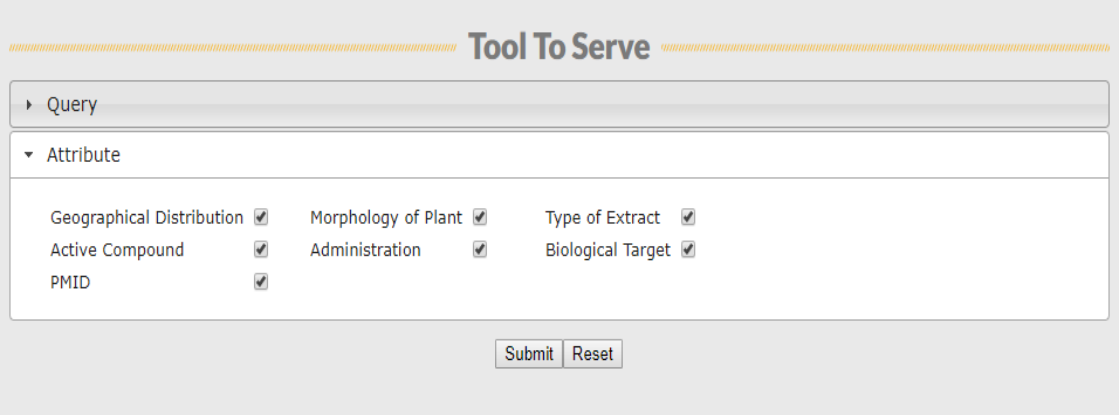

Fig. 5. Selection of attributes in the front end search window at MED-PDB

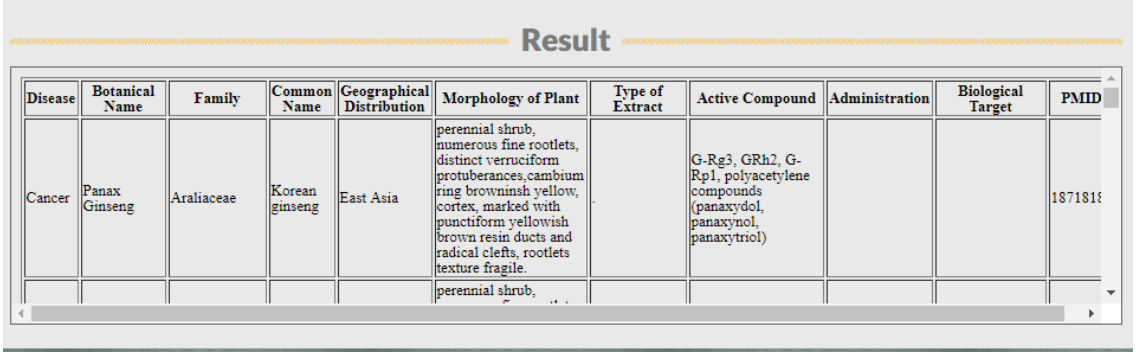

Fig. 6. Search result for the desired input (Cancer) showing description about selected attributes generated by Back End Flat file database.

The MED-PDB is a cachet that has collated the literature on medicinal plants with the diseases. By permitting a user to swiftly overlay the earlier observed correlations, we have made it plausible to give meaning to active compound of plant extract in a clinical context, helping usher both clinical and potential treatment of possibly severe disease on an individual basis.

9. Conclusion

This report has delineated the concept of MED-PDB, and conferred some of its salient aspects. The MED-PDB has given a new dimension to researchers who are looking for various aspects of plants, medicinal properties and their active compounds which are used in therapeutics of disease. MED-PDB will foster sharpened and quick availability of the information rather than setting up an individual data centre for each plant or its various properties as done earlier.

The above mentioned information is the legitimate result of manually curetting and studying various research papers reviewed at available database at NCBI. MED-PDB database saves a lot of money and time of researchers as the data from different sources is present in a single database. In the absence of MED-PDB database, researchers might have to invest a huge amount of money and time for getting information about various plants and related aspects mentioned in the database.

MED-PDB database compiles a good number of attributes of medicinal plants which can serve as a basis for research prospects. MED-PDB has given the latitude to access the information from anywhere without any circumscription of purchasing policy etc. which makes it a powerful tool.

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