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A Comparative Study on Non-Surgical Treatment of Leg Pain due to Chronic Exertional Compartment Syndrome and Lateral Compartment Arthritis by the Lotus Posture "Padmasana"

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Abstract

Leg pain with or without Knee joint pain is a very common symptom in adult age groups and especially in those who are athletes, recreational runners, elite runners and military recruits. The Chronic Exertional Compartment Syndrome (CECS) and Lateral Compartment Arthritis (LCA), comprises most number of the cases in adults. Though various non-surgical modes of treatment like oral analgesics, unloader bracing, shoe modification etc are available for these, none of them have controlled trials to verify the efficacy of the same. From the rich ancient knowledge of India, a yoga position - the" Lotus" (Padmasana), have been tried in the above mentioned cases. With 84.6% of success rate within two months of regular practice, Padmasana offers an effective approach to deal with the cases of CECS and LCA.

Keywords: Padmasana (Lotus Posture), Knee pain, Leg pain, Lateral Compartment Arthritis (LCA), Chronic Exertional Compartment Syndrome (CECS), Genu valgum

Introduction

The Lotus position (Padmasana) is a crossed-legged sitting asana (Figure 1) originating in meditative practices of ancient India, in which the feet are placed on opposing thighs. It is an established asana in Hindu Yoga, Jain and Buddhist contemplative traditions, adopted to allow the body to be held completely steady for long periods of time. Sciatica, sacral infections and injured knee are contraindications to attempting the asana.[1]

Figure 1: The Lotus Position (Padmasana), demonstrated by eminent Yogi Lahiri Mahasaya

The most common form of presentation of adult cases of leg pain, with or without the involvement of knee joints are CECS and LCA. With various non-surgical modes of available treatments, conclusive evidence of efficacy is lacking.

Padmasana, the Lotus Posture, helps to diminish the pain of leg and knee joint related to CECS and LCA. Due to exactly opposite mechanical position of the structures of knee, assumed during the Asana (posture), the Lotus posture may help in delaying the process of genu valgum.

Background and History

Patients with LCA of the knee joint typically have pain and grinding that are localized to the lateral aspect of the knee. Patellofemoral symptoms may or may not be present, depending on the degree of degenerative change. With advanced disease and deformity, patients notice a valgus orientation of the knee joint (Figure 2).

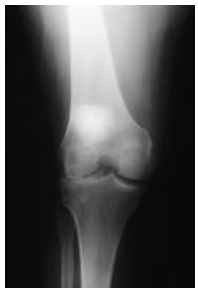


Figure 2: Radiographic evidence of erosion of lateral cartilage of the knee resulting in valgus deformity, in a case of LCA.

Arthritic destruction of the lateral compartment of the knee manifests as a genu valgum deformity. A valgus deformity is defined as a malalignment that exceeds the normal 7°-10° femorotibial angulation. With progressive valgus malalignment , the medial soft tissue of the knee joint stretch, whereas the lateral soft- tissue structures of the knee , including the lateral collateral ligament , iliotibial band , and lateral capsule contract. Over time these deformities become fixed.

The following non-surgical modes of treatment are being in use with no controlled studies to verify the efficacy of this approach, in patients with mild deformities: oral analgesics, aerobic conditioning of the lower extremity muscles. unloader bracing, shoe modification, activity modification, weight reduction and use of cane during ambulation.

Surgical modes of available treatments are corrective osteotomy and total knee replacement (TKR)[2].

According to American Medical Association, more than 10 million Americans have knee osteoartritis. The condition is recognized as one of the 5 leading cause of disability in non-institutionalized adults by the Centers for Disease Control and Prevention (CDC).

CECS is related to marked increase in tissue pressure within the confinement of closed fascial space during exercise and or progressive muscle activity, leading to rise in the intra-compartmental pressure and thereby impaired muscle perfusion.[3-5]

Deoxygenation of muscle results in increased permeability, causing shift of fluid in the interstitial space. Compromise of the microcirculation leading to ischemia, ultimately resulting in pain. 95% of cases of CSES occur in anterior and lateral compartments of the leg. CSES occurs most commonly in young adult recreational and elite runners, military recruits, and athletes.

Materials and Method

39 patients having leg pain with or without knee pain due to Lateral Compartment Arthritis (LCA) and Chronic Exertional Compartment Syndrome (CECS) have been selected in the age group of 21- 63 years. Post operative cases and those having sciatica, history of knee injury and sacral infections have been excluded from the study group.

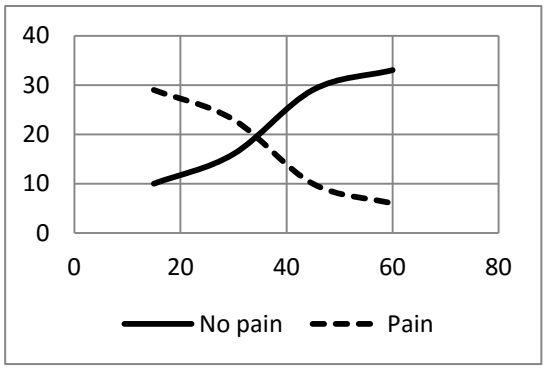
Initially all the subjects have been instructed and demonstrated the position of padmasana. A 15 minute session was arranged every day at first, which later was increased upto 30 minutes over a period of 2 months. Depending on the tolerability of the patients, experienced individually, the patients have been allowed to assume the position of padmasana for as minimum as 15 seconds to the maximum possible, uptill 30 minutes. Symptoms of the patients were monitored every 15 days.

Results

The result shows that 33 patients (84.6%) out of 39 have noticed improvement of their symptom of leg and knee pain. Only 06 patients (15.4%) have been referred for super-specialty check-up, to be diagnosed for the exact pathology and or associated disease as cause of their symptoms, overlooked yet.

Table 1: Number of Patients undergoing Padmasana suffering from LCA and CECS

Symptoms	Number of Patients undergoing Padmasana			
	15 Days	30 Days	45 Days	60 Days
No pain	10	16	29	33
Pain	29	23	10	06



Discussion

The mentioned benefits of Padmasana in the ancient literature are mainly oriented to spiritual science and in meditation. It's medical benefits for physical well being and healing process is not well described in the available literatures.

The locked position of the legs in the posture of padmasana helps to decrease the intracompartmental pressure of the muscle groups. This improves the microcirculation when the legs are made straight after finising the posture of padmasana.[6-9]

Owing to the typical position of the knee assumed during Padmasana, the intercartilagenous

space of anterior and lateral portion of the knee joint widens between the apposing cartilagenous surfaces of femur and tibia. As many of the pathology of knee joint pertain to diminution of intercartilagenous space between femur and tibia, it can be anticipated that assuming a position of Padmasana, will favor the required structural position of the components of knee joint. In other words, the position of knee assumed during Padmasana will counter and delay the process of lateral bending of knee (genu valgum, Figure 3).

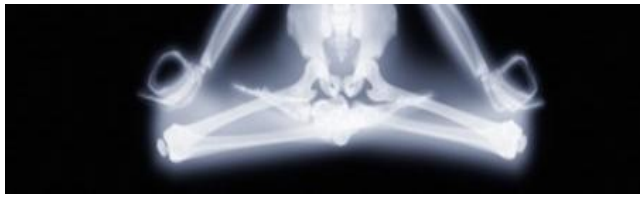


Figure 3: Radiographic image of mechanical advantage of the posture of Padmasana, showing increase in space within the knee joint. The patella also assumes a relaxed state.

Increase in intercartilagenous joint space will improve microcirculation to foster the healing process. There will be improved oxygenation and better washout of locally produced free radicals.

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Study of the Survival of Indicator Organisms *Escherichia coli* and *Enterococcus* Spp. in Source Separated Human Urine to Evaluate Microbial Health Risk Associated with its Reuse as Fertilizer

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Abstract

In this study the survival of two important indicator bacteria *E. coli* and *Enterococcus* spp in source separated human urine was studied. The urine samples were obtained from urine diverting toilets installed in different households situated at Siddhipur, Nepal. Urine samples so obtained were all stored in air tight plastic drum for a period of several weeks to several months prior to sampling time. The pH of urine was in the range of 8.8 to 9.0. Number of bacteria per 100 ml of urine was calculated. Enumeration was done by taking an average count of three plates. Total of five samples were analyzed. *Enterococcus* spp was isolated from all the samples, the highest number being 2500 and the lowest being 200 with the average of 1250. On the other hand *E. coli* was found to be absent in all of the samples. The result obtained suggests that gram negative *E. coli* had a rapid die-off rate as compared to gram positive *Enterococcus* spp.

Keywords: Human urine, Hygiene risks, Fertilizer, *E. coli*, *Enterococcus*, Inactivation, Microbial persistence.

Introduction

Human excreta contain plant nutrients and have been used for crop fertilization in many countries[1,2]. Human urine owing to its nutritional value could be considered as a good plant fertilizer but at the same time its use can involve microbiological risk as human urine is not totally sterile[3]. The risks include the presence of possible pathogens via contamination with faeces as faeces contain high amount of enteric microorganisms including many pathogens and opportunistic pathogens and involves greater health risks if reused[1,3] or excretion of pathogens from diseased humans4. The pathogens known to be excreted in urine *are Leptospirainterogans, Salmonella typhi, Salmonella paratyphi*, and *Schistosomahaematobium*[5]. The use of human urine in crop fertilization could therefore establish new transmission routes for disease infection for the person involved in the application work[4]. The occurrence of pathogenic micro-organisms in source-separated urine because of faecal cross-contamination has been reported[6]. Hence any faecal cross- contamination that may occur by misplacement of faeces in the urine separating toilet is regarded as a possible health risk[1,7].

In most survival studies with human urine *E coli* and *Enterococcus* spp have been used as

indicator organisms and these two organisms are among the two important indicator bacteria that have been used to infer the presence of other potentially harmful pathogens[8]. *E. coli* is one of the major cause for the death due to enteric infection[9]. Comparatively gram negative *E. coli* have faster inactivation than positive enterococci[1]. The fate of the enteric pathogens entering the urine tank is of vital importance for the health risks related to the handling and reuse of the urine. To ensure its use as a fertilizer, therefore it is necessary to estimate the survival of various microorganisms in urine[1]. Many of the studies that have been conducted in the past to examine rates of indicator bacteria inactivation have made use of laboratory-cultured inoculants and very few studies have incorporated the use of inoculants of the greatest concern to managers, such as sewage, or urban run-off. This could be important as the flora in these inoculants can vary greatly from laboratory-cultured –strains, which may lead to very different inactivation rates in natural systems[8]. Therefore in this study we have used source separated human urine from urine diverting toilet as an innoculant. The study presented here is an explorative rather than confirmatory to determine the persistent of two important indicator bacteria *E. coli* and *Enterococcus* spp in source separated human urine.

Materials and Methods

Sample collection:

The urine samples from urine diverting toilets which was collected and stored in a air tight plastic drum for a period of several weeks to several months prior to sampling was transported in a air tight sterilized bottles to the Microbiology lab for bacteriological analysis and kept at 4 °C prior to analysis. The urine in the drum was stirred well while sampling.

Isolation & enumeration of target organisms:

For the isolation and enumeration of both *E. coli* and *Enterococcus* spp, membrane filter method was used. Each time before proceeding for the isolation appropriate dilution of the urine sample was made (2 ml of sample was diluted with 98 ml of sterile distilled water so that the total volume becomes 100 ml). Isolation was made by making use of selective media (Mac Conkey agar for *E coli* and bile aesculine azide agar for Enterococcus spp).The plates containing the membrane with bacteria were incubated at a temperature of 44 °C for 24 hours. The colonies developed after incubation were then counted and the average of the triplicate microbial counts were determined and results were expressed per 100 ml of sample by applying the formula:

Number of bacteria per 100 ml of sample = (Colony count / Volume of sample used) x 100

Identification of organism

Pure culture of the typical colonies were formed on Nutrient agar and the pure cultures were then again grown in the respective selective medias and subjected to Gram staining and various biochemical tests for further identification.

Identification of Enterococcus spp:

Following tests were performed for the identification of *Enterococcus* spp:-Gram staining, Aesculin hydrolysis test, Litmus milk reduction test, Catalase test.

Identification of E. coli

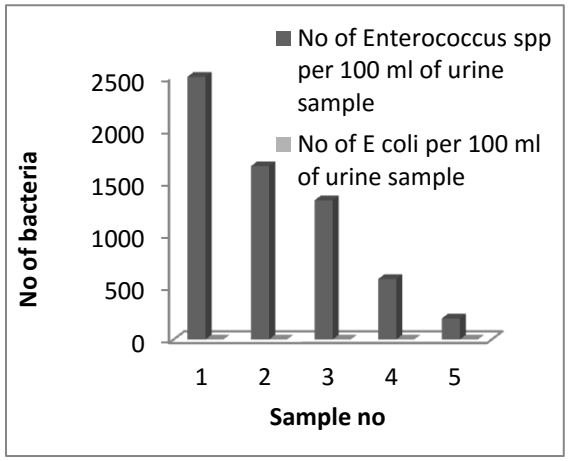
Following tests were performed for the identification of *E. coli*:-Gram staining, IMViC test, TSIA test.

Result and Discussion

As is shown in the Table 1 *Enterococcus* spp was isolated from all the urine samples where as *E. coli* remained absent in all of the samples. This suggests that gram negative *E. coli* is rapidly inactivated whereas gram positive *Enterococcus* spp is more persistent. Our result is in agreement with those presented by Hoglund et.al, 1998, Schonning et.al, 2002, Vinneras et.al, 2008 and Chandran et.al, 2009 [2,6,10,11]. The rapid inactivation of E coli could be related to the increase in pH level of around 9 and the organism might not be suitable for indicating faecal contamination due to its rapid die off [2,12].

TABLE 1: Numbers of *Enterococcus* spp and *E. coli* per 100 ml of stored human urine

No. of *Enterococcus* spp per 100 ml of urine sample	No. of *E coli* per 100 ml of urine sample
2500	0
1650	0
1325	0
575	0
200	0



The handling and reuse of all different types of waste products with human or animal origins involve hygiene risks and human excreta whether faeces or urine when reused might contain enteric pathogens able to cause infection by consumption of crops that have been fertilized[1]. Although urine contains few enteric microorganisms, faeces on the other hand always contain high amounts of enteric microorganisms including many pathogens and opportunistic pathogens. The urine fraction utilized in agriculture for human food plants must in that case be free of faeces[3]. In summary, the question whether the reuse of human urine on agricultural land is suitable is one of the key questions. So far, the level of risk that is acceptable is unknown. Therefore the acceptable risk will be one of the main factors determining the future utilization of source separated human urine in agriculture[1].

Acknowledgement

We wish to thank Eawag, Swiss Federal Institute of Aquatic Science and Technology for providing us the fund to carry out this research project. We extend our sincere thanks to Khatmandu University for helping us with lab facilities. Our special thanks go to Mr. Bastian Etter and Elizabeth Tilley for their great help during the entire project.

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Validated HPTLC Method for the Determination of Azelastine hydrochloride in Bulk Drug and Dosage Form

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Abstract

A simple, precise, and accurate HPTLC method has been developed and validated for estimation of azelastine hydrochloride in the bulk drug and in tablet dosage forms. The stationary phase was aluminum plates precoated with silica gel 60 F254, and the solvent system consisted of toluene : chloroform : methanol (5 : 4 : 2 v/v/v). Densitometric evaluation was performed at 212 nm. The Rf value was found to be 0.41±0.02. The linearity of the method was found to be in the range of 40-140 ng/spot. The lower limit of detection was 20 ng/spot. The method was found to be accurate and precise and was validated as per ICH guideline.

Keywords: Azelastine hydrochloride, HPTLC, estimation, validation

Introduction

Azelastine hydrochloride chemically, (RS)-4-[(4-chlorophenyl)methyl]-2-(1-methylazepan-4-yl)-phthalazin-1-one, having various pharmacological actions such as, lipoxygenase inhibition, platelet aggregation inhibition, bronchodilation, anti-allergic, histamine H1 antagonist (non-sedating)[1]. As an antihistaminic agent, it competes with histamine for the H1-receptor sites on effector cells and acts as an antagonist by inhibiting the release of histamine and other mediators involved in the allergic response. There are very few methods have been developed for the estimation of Azelastine hydrochloride in bulk drug and formulation using HPLC [2,3]. There are some LC-MS/MS methods are also available for the estimation of Azelastine hydrochloride in biological matrix[4,5]. To best of our knowledge, there has been no published HPTLC method on the estimation of Azelastine hydrochloride in bulk drug and formulation. Therefore, the main objective of this research work is to develop a simple, precise and robust HPTLC method for the estimation of Azelastine hydrochloride in bulk drug and formulation. The developed method was validated as per ICH guidline Q2 (R1)[6].

Methodology

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 F254 silica gel, [20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany] using a Camag Linomat V (Switzerland) sample applicator. The mobile phase consisted of

toluene : chloroform : methanol (5 : 4 : 2 v/v/v). The development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). The optimized chamber saturation time for the mobile phase was 40 min at room temperature (25 °C ± 2) at relative humidity of 60 % ± 5. A 1000 µg/mL standard stock solution was prepared in acetonitrile for azelastine hydrochloride. From the standard stock solutions, diluted standard solutions were prepared containing 100 µg/mL for azelastine hydrochloride. The stock solution was stored at 2-8 °C protected from light. Validation of the optimized TLC method was carried out with respect to the linearity, accuracy, precision, robustness and specificity. The content of azelastine hydrochloride was tested in conventional eye drops (Brand name: Azelast, Batch No. HKJ0595, Label claim: 0.5 mg Azelastine Hydrochloride per mL, Manufactured by: Unimed Technologies Ltd., Gujrat, India), 1mL of eye drops was taken which is equivalent to 0.5 mg of Azelastine Hydrochloride.

Result and Discussion

A typical densitogram of standard azelastine hydrochloride using the toluene : chloroform : methanol (5 : 4 : 2 v/v/v) for TLC is given in Figure 1. The results of the validation parameters of this study were summarized in Table 1.

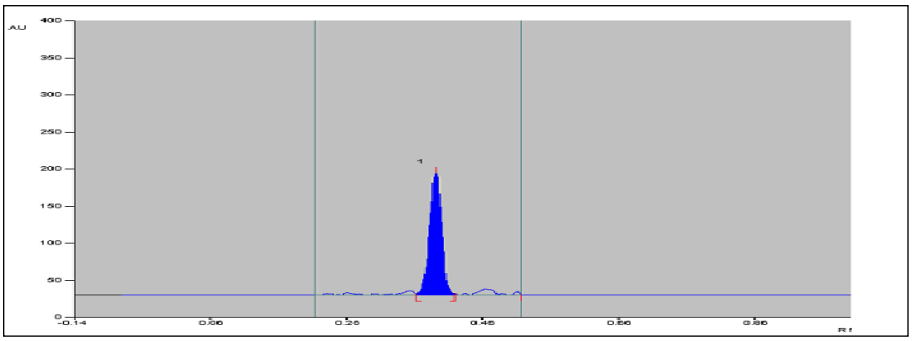

Figure1. HPTLC densitogram of standard Azelastine hydrochloride (100µg/mL)

Table 1. Summary of the validation parameters

Validation Parameters	Results
Linearity	40 – 140 ng/spot
Limit of Detection (LOD)	20 ng/spot
Limit of Quantitation (LOQ)	40 ng/spot
Precision	% RSD < 2 %
Accuracy (Recovery)	98.88 - 100.90 %
Specificity	Method was found to be Specific
Robustness	Method was found to be robustness

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Introducing Open Patent in Drug Discovery and the Need of Federation of Open Drug Discovery (FODD) or United Open Source Drug Discovery (UOSDD) for Unified Approach

Soumendranath Bhakat

Co-Founder Open Source Drug Design and In-Silico Molecules

Abstract

Open Source Drug Discovery (OSDD) initiatives is one of the most emerging new approach in drug discovery with a motto of affordable healthcare. But in some cases the motto of “Open Source” and “Openness” get hampered because of faulty licensing strategy and lack of unified approach. Often patent and copyright creates some fundamental problem in front of Open Source Drug Discovery whose ideal motto should be free distribution, adaptation, remix and non-restricted flow of data. This paper proposes a new concept of “Open Patent” in drug discovery and need of a unified forum of all OSDD initiatives to foster fast innovation with a true “Open Science” approach for affordable healthcare.

Keywords: Open Source Drug Discovery; Patent; Copyright; Copyleft; Open Patent; Federation of Open Drug Discovery (FODD) or United Open Source Drug Discovery (UOSDD)

Introduction

Open Source Drug Discovery (OSDD) is a generic term whose motto is to develop new drug/ drug-like candidates with collaborative and open source approach for an affordable healthcare. Open Source Drug Discovery in broad sense a concept, practice and movement which ideally should believe in free flow of data sharing and collaboration for affordable healthcare.

The majority of Open Source Drug Discovery projects working under same or different name such as CSIR-Open Source Drug Discovery[1], The Synaptic Leap[2], Usefulchem[3], Open Source Malaria/OSDDMalaria & OpenWetWare community[4][5], Open Source Drug Design and In-Silico Molecules[6] etc. having same motto of affordable healthcare. When CSIR-OSDD uses OSDD license[7] which has a huge restriction on free data sharing and free reproduction, adaptation, or distribution of datas; the other ventures mainly uses different versions of creative commons license[8] with some added clauses in some cases. OSDD initiatives adapting globally accepted creative commons license encourages a free spirit in data sharing with proper credit to all cross platform collaborators. Apart from this it is widely believed that OSDD projects in general will create problems in copyright and patent policy.

With the emergence of Open Source as a trend the debate over patent, copyright in innovations just intensified dramatically. Especially in drug discovery the patent plays a significant

role in creating market monopoly, high price drugs and restricts further distribution, adaption, remix and sharing of work which ultimately affects innovation [9].

Patenting and Copyright in Open Source Drug Discovery initiatives are fundamentally incompatible and the issue of patenting and copyright are the biggest threat towards Open Source Drug Discovery. But the word “Patent” and “Copyright” able to build a permanent place in the soul of a great number of researchers that they were unable to think beyond these two words. The new “Open Patent” and “Copyleft” approach will try to co-ordinate between patent, copyright and open source to ensure free distribution, remix, adaptation, sharing, commercial or non-commercial usage for greater human welfare.

Another major issue with different OSDD ventures is that there is almost no collaboration between different OSDD ventures which ultimately slows down overall development process and this negative attitude and conflict of interest among each other in Open Source Drug Discovery ecosystem is hampering the growth of new OSDD ventures in terms of data sharing, technology transfer, funding, collaboration which ultimately affects innovation. Hence there is a need of is a need of an unified governing body quite similar to that of United Nations [10], which will co-ordinate between all OSDD initiatives across the world and will govern that the motto of Open Source and will ensure that all resources across different platform is well coordinated and will ensure that the motto of Open Source really serves. Thus the unified governing body of OSDD ventures will be named as Federation of Open Drug Discovery (FODD) or United Open Source Drug Discovery (UOSDD) which will govern the whole OSDD ventures and will act as a patriarch of Open Patent license in drug discovery.

Introducing the Idea of Open Patent in Drug Discovery

The term “Open Patent” is quite an emerging trend in terms of software and related inventions in which “Open Patent” movement seeks to build a portfolio of patented inventions that can be freely distributed under a “Copyleft” like license [11][12][13].

The “Open Patent” idea in Drug Discovery will have some twist and will try to co-ordinate between “Openness” and “Patents/Copyright”.

Some initial proposals as clauses of Open Patent in Drug Discovery:

1. Under Open Patent innovation(innovation that is Open Patented) the innovators of a drug/drug-like candidate will get copyright and a lifetime patent of the invention but the invention will be free for distribution, modification, sharing and it requires that any resulting copies or remix works follow the same rule or must be Open Patented.

   For example, An OSDD platform discovers a new drug like candidate or drug molecule and adapt the Open Patent license. That will permanently ensure the copyright or patent of that discovery with that particular OSDD venture (it may be any other organization also) or its contributors (copyright and a lifetime patent will be there with all members of research group/s who discovers a new drug candidate). But the molecule/drug will be free for

adaptation, distribution, reproduction, modification providing that any resulting copies should also adapt the policy of Open Patent and should be free for adaptation, distribution, reproduction and modification.

2. Whenever a company will take a drug/molecule which is Open Patented under "Open Patent License" for further development process such as clinical trial, production, marketing etc. the Federation of Open Drug Discovery (FODD) or United Open Source Drug Discovery (UOSDD) will oversees all the development process starting from clinical trial till marketing and beyond to quality and standard of Open Patented products. FODD or UOSDD will calculate the price of a drug which is Open Patented in a manner based on buying capability of people from specific region and will set the price such that the people having lowest buying power can afford to purchase a newly discovered drug. Any company/organization can market same molecule but must follow the controlled price order decided by FODD or UOSDD to ensure affordable healthcare.
    a. Conflict scenario 1: Suppose company X makes an oral suspension of a drug molecule which is already Open Patented and get a new Open Patent license for that oral suspension. Suppose after 2 days another company Y comes up with a same/similar oral suspension of that drug molecule. In this case the company Y will not get an open patent as company X already gets an open patent for the same thing. But company Y can market the product obeying the controlled price decided by FODD or UOSDD. Thus the market monopoly of a particular company/organization will not exist and poor people will have a option to choose and open up new alternatives.
    b. Conflict scenario 2: Suppose company/organization developed a new derivative molecule/drug which uses an existing drug/molecule which is already open patented. In this case the company must Open Patent its new molecule/drug with proper credit to the previous Open Patented innovation (giving proper reference in all publications and documents). Once Open Patented the company/organization is free to market the new drug molecule (after getting approval as a drug) and must follow the controlled price specified by FODD or UOSDD. Thus the cycle of innovation, patenting and free sharing, distribution, adaptation will continue.
    c. Conflict Scenario 3: After taking the "Open Patent" license a research organization can ask a company to look after the marketing of an Open Patented drug, in that case the marketing company must obey the controlled price decided by FODD or UOSDD.
3. All raw datas of the innovation or discovery process holding "Open Patent License "must be available freely in public domains and advised to be published in Open Access Peer Reviewed journals.
4. Granting the Open Patent license will be done by FODD or UOSDD after an internal evaluation consulting all members of FODD or UOSDD and then by a public discussion to ensure credibility of datas.

5. The Open Patent License will be valid for lifetime.
6. The pricing of Open Patented drugs/products will be controlled by FODD or UOSDD based on judging the buying power of a people of a specified region and will the fix the price such that poor/lowest income people can afford the drug/product (the FODD or UOSDD will invite public discussion in setting up the price based on different region).
7. The Open Patent license can be revoked if public discussion at later stage find some faults in terms of data fabrication, conflict among collaborators(in cases where proper credit is not given to a person who contributed to the project) etc. FODD or UOSDD will charge an amount equivalent to the whole discovery process till clinical trial.
8. Once it has been found that companies/organizations marketing an Open Patented molecule ignoring the controlled price mentioned by the FODD or UOSDD in these cases FODD or UOSDD can charge a maximum amount equivalent to the anticipated whole market price of that molecule in 1 year.
9. All amount generated from these punishments will be used to nurture all OSDD ventures and supporting the budding OSDD ventures.
10. All conflict scenarios will be judged in a three way policy involving the FODD or UOSDD community, the accused company/organization/inventor and public discussion. Based on majority of opinion the decision will be taken.
11. The decision again can be challenged. In that case FODD or UOSDD and accused party will nominate a neutral panel and upon judgement of neutral panel and public discussion the decision will be taken.
12. Open Patented drugs are free for marketing at a lower price than that of the controlled price.

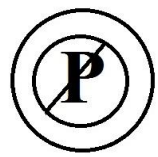

Figure 1: The proposed logo of Open Patent in Drug Discovery

Introducing the Idea of Federation of Open Drug Discovery (FODD) or United Open Source Drug Discovery (UOSDD) [14]

Why there is a need of this type of unified forum and how it will work:

1. Take for example there are different forums dealing with same category of molecule. For example there are 3-4 different OSDD initiatives dealing with small molecule drug discovery related to malaria. They have their own databases and it is very rare that they cross check their database with other OSDD platforms as it is quite tough because of huge

submission of datas by its users. Hence it is quite possible that a huge amount of resource can be wasted to develop the same molecule with same technique. In this type of scenario the FODD or UOSDD will look after the whole programmes across different cross platforms and in this type of conflict scenario it will appeal both OSDD initiatives to collaborate together so that a unified open approach can be taken for discovery of a small molecule.

2. The FODD or UOSDD will look after the funding issue as per all OSDD project concerns. Based on requirement of different OSDD portfolios across time OSDD will raise fund and distribute it across all members after proper consideration and discussion with all OSDD platforms.
3. FODD or UOSDD will look for industry partner which is a quite a huge problem in terms of small molecules come from any OSDD initiative. FODD or UOSDD will arrange industry partner for future production, clinical trial and other development processes that will be needed for drug-like molecules that will come from different OSDD initiatives. FODD and UOSDD will ensure and look after that all drug-like molecules that will come from different OSDD initiatives go through a smooth development process.
4. It is always true that all OSDD initiatives lack in different technical fields either it is different features regarding to its website, different aspects of formatting the existing feature, different aspects of sharing datas et al. The FODD or UOSDD will contribute with different suggestions on time to time basis to all OSDD platforms to upgrade its professional value and will promote all OSDD initiatives.
5. FODD or UOSDD will look after different OSDD initiatives and will ensure that the motto of Open Source and Open Culture really in practice in those OSDD ventures.
6. **The Proposed Organization Structure of FODD or UOSDD**
    a. Board of Directors of FODD or OSDD
    b. Founders/Project Leaders/CEOs of All Current and Emerging OSDD initiatives.
    c. Industrial Consultants who have previous research experience in different drug discovery and NCE development who will support all OSDD ventures with their valuable tips during the synthesis, clinical trial and production and finding industrial partners
    d. Academic consultants who will help with different academic and technical tips to all OSDD initiatives.
    e. Volunteers of Open Science & Culture Motto who will ensure that the motto of Open Science & Culture really serving across different platforms and will report the Board of Directors, OSDD Members with their tips and recommendations.
    f. Financial Division: Will raise funds which will be distributed to different OSDD ventures depending on their status after a proper discussion with all new and existing members of community. It will also raise money from donation and will

strategize different business model related to OSDD to raise fund for OSDD initiatives keeping the Open Source motto intact.

g. The persons from Law background to look after all issues related to Open Patent and other legal issues within the interest of FODD or UOSDD.

7. FODD or UOSDD will take all necessary actions regarding Open Patent and related issues and will look after all technical and legal aspects of Open Patent .FODD or UOSDD will work on topics related to license of Open Patent, licensing strategy of OSDD initiatives and other continuous developments to enrich Open Source. FODD or UOSDD will recommend all OSDD ventures to adapt Open Patent in innovation will strongly work forward to make it happen.
8. FODD or UOSDD will ensure all existing OSDD ventures will support new and emerging OSDD initiatives with their existing capacity, excess fund, synthesis expertise and consultancy.

Conclusions

Open Source Drug Discovery is emerging new approach for fast innovation and affordable healthcare. The Open Patent and FODD or UOSDD are just only ideas which might change the dynamic of the OSDD ventures in a more positive way and will serve the best interest of “Openness” and affordable healthcare. It is a crunch situation for all current and emerging OSDD initiatives and a unified approach is predicted to serve the motto of affordable healthcare, crowd-sourcing and a fast innovation in a more efficient way.

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Banned Drugs

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In India the Drugs and Cosmetics Act, 1940 regulates the import, manufacture, distribution and sale of drugs and cosmetics. Section 26A was inserted in the Act by the Drug and Cosmetics (amendment) Act, 1982 (68 of 1982) with effect from 01.02.1983 empowering Central government to prohibit manufacture, etc. of any drug and cosmetic in public interest. This section was not there in the original Act of 1940. Thus, before 1st February 1983 there was no provision for prohibiting the manufacture, sale or distribution of any drug which involved any risk to human beings or animals; or that did not have the therapeutic value claimed or purported to be claimed or contained such ingredients in such quantity for which there was no therapeutic justification. Therefore, this was a landmark amendment in the Drugs and Cosmetics Act to ensure safety and efficacy of medicines being manufactured for sale or sold or distributed in India.

Section 26A reads as under:

"26A. *Power of Central Government to prohibit manufacture, etc., of drug and cosmetic in public interest.* — Without prejudice to any other provision contained in this Chapter, if the Central Government is satisfied, that the use of any drug or cosmetic is likely to involve any risk to human beings or animals or that any drug does not have the therapeutic value claimed or purported to be claimed for it or contains ingredients and in such quantity for which there is no therapeutic justification and that in the public interest it is necessary or expedient so to do, then, that Government may, by notification in the Official Gazette, prohibit the manufacture, sale or distribution of such drug or cosmetic."

Utilizing this power Central government banned 22 drug products on 23rd July 1983 and thereafter from time to time manufacture for sale, sale and distribution of many other drugs has been prohibited by subsequent notifications in The Gazette of India, part II – Section 3 -Sub-section (i). All such Notifications issued hitherto are tabulated below:

G.S.R.	Date of notification	Drug
578(E)	23.07.1983	1. Amidopyrin. 2. Fixed dose combinations of Vitamins with anti-inflammatory agents and tranquilisers 3. Fixed dose combinations of atropine inAnalgesics and anti-pyretics. 4. Fixed dose combinations of Strychnine and Caffeine in tonics. 5. Fixed dose combinations of Yohimbine and Strichnine with

G.S.R.	Date of notification	Drug
		Testosterone and Vitamins. 6. Fixed dose combinations of Iron with Strichnine, Arsenic and Yohimbine. 7. Fixed dose combinations of sodium Bromide/ chloral Hydrate with other drugs. 8. Phenacetin. 9. Fixed dose combinations of anti-histaminics with anti- diarrhoeals. 10. Fixed dose combinations of Penicillin with Sulphonamides. 11. Fixed dose combinations of Vitamins with Analgesics 12. Fixed dose combinations of Tetracycline with Vitamin C. 13. Fixed dose combinations of Hydroxyquinoline group of drugs except preparations preparations meant for external use. (substituted by G.O.I. notification No.G.S.R.793(E) dt.13.12.1995.) *14. Fixed dose combinations of corticosteroids with any other drug for internal use. *15. Fixed dose combinations of Chloramphenicol with any other drug for internal use. 16. Fixed dose combinations of crude Ergot except those containing Ergotamine, caffeine, analgesics, antihistamines for the treatment of Migraine headaches. 17. Fixed dose combinations of Fixed dose combinations ofitamins with antiT.B.Drugs except combination of Isoniazid with Pyridoxin Hydrochloride (Vitamin B6.) 18. Penicillin skin/eye ointment. 19. Tetrracycline liquid oral preparations. 20. Nialamide. 21. Practolol. 22. Methapyrilene, its salts. * substituted by G.O.I. notification G.S.R.1057(E) dt 03.11.1988
49(E)	31.01.1994	23. Methaqulone.
322(E)	03.05.1984	24 Oxytetracycline Liquid Oral Preparations. 25 Democlocycline Liquid Oral Preparations.
863(E)	22.11.1985	26. Combinations of Anabolic steroids with other drugs.
700(E) 743(E)	15.06.1988 10.08.1989	27. Fixed dose combination of Oestrogen and Progestin(other than oral contraceptives) containing per tablet oestrogen content of more

G.S.R.	Date of notification	Drug
		than 50 mcg.(equivalent to Ethenyl Estradiol) and progestin content of more than 3mg(equivalent to Norethysterone Aceate and all fixed dose combination injectable preparations containing synthetic oestrogen and progesterone.
999(E)	26.12.1990	28. Fixed dose combination of Sedatives/hypnotics/anxiolytics with analgesic–antipyretics. 29. Fixed dose combination of Pyrazinamide with other anti tubercular drugs except combination of Pyrazinamide with rifampicin and INH as per recommended daily dose given below: - Minimum Maximum. Rifampicin 450 mg 600 mg INH 300 mg 300 mg Pyrazinamide 1000 mg 1500 mg. 30. Fixed dose combination of histamin H2 – receptor antagonists with antacids except those combinations approved by the Drugs Controller, India. 31. The patent and proprietary medicines of fixed dose combinations of essential oils with alcohol having a percentage higher than 20% proof except preparations given in the Indian Pharmacopoea. 32. All Pharmaceutical preparations containing Chloroform exceeding 0.5% w/w or whichever is appropriate.
69(E)	11.02.1991	33. Fixed dose combination of Ethambutol with INH other than the following: INH Ethambutol 200 mg 600 mg. 300 mg. 800 mg. 34. Fixed dose combination containing more than one antihistamine. 35. Fixed dose combination of anthelmintic with cathetric/ purgative except for piperazine. 36. Fixed dose combination of salbutamol or any other drug having primarily bronchodialatory activity with centrally acting anti-tussive and/or antihistaminic. (Notified vide G.S.R. *290(E) 16.04.2008)* 37. Fixed dose combination of laxatives and/or anti-spasmodic drugs in enzyme preparations. *38. Fixed dose combination of Metaclopromide with other drugs

G.S.R.	Date of notification	Drug
		except for preparations containing Metaclopromide and aspirin/ paracetamol. 39. Fixed dose combination of centrally acting anti-tussive with antihistamine having high atropin like activity in expectorants. 40. Preparations claiming to combat cough associated with asthma containing centrally acting anti-tussive and/or other antihistamine. 41. Liquid oral tonic preparations containing and/or otherphosphates and/or central nervous system stimulant and such preparations containing alcohol more than 20% proof. 42. Fixed dose combination containing Pectin and/or Kaolin with any drug which is systemically absorbed from GI tract except for combinations of Pectin and/or Kaolin with drugs not systemically absorbed. * substituted by G.O.I. notification No.G.S.R.603(E) dt.24.08.2001
304(E)	07.06.1991	43. Chlora Hydrate as a drug.
111(E) and corrected 612(E)	22.02.1994 09.08.1994	44. Dover's Powder I.P. 45. Dover's Powder Tablets I.P.
731(E)	30.09.1994	46. Anti diarrhoeal formulations containing Kaolin or Pectin or Attapulgite or activated charcoal. 47. Anti diarrhoeal formulations containing Phthalyl sulphathiazole or Sulphaguanidine or Succinyl Sulphathiazole. 48. Anti diarrhoeal formulations Neomycin or Streptomycin or Dihydrostreptomycin including their respective salts and esters. 49. Liquid oral anti-diarrhoeals or any other dosage form for paediatric use containing Diphenoxylate or Loparamide or Atropine or Belladonna including their salts or esters or metabolites Hyoscyamine or their extracts or their alkaloids. 50. Liquid oral anti-diarrhoeals or any other dosage form for paediatric use containing halogenated hydroxyquinolines. 51. Fixed dose combination or anti-diarrhoeals with electrolytes.
57(E)	07.02.1995	52. Patent and Proprietary Oral Rehydration Salts other than those conforming to the following parameters:

G.S.R.	Date of notification	Drug
		(a) Patent and Proprietary Oral Rehydration Salts on reconstitution to one liter shall contain: - Sodium – 50 to 90 millimoles. Total osmolarity – not more than 290 millimoles. Dextrose:Sodium molar ratio- not less than 1:1 and not more than 3:1. (b) Patent and Proprietary cereal based Oral Rehydration Salts on reconstitution to one liter shall contain: - Sodium – 50 to 90 millimoles. Total osmolarity – not more than 290 millimoles. Precooked rice - equivalent to not less than 50 gms. And not more than 80 gms. As total replcement of dextrose. (c) Patent and Proprietary Oral Rehydration Salts (ORS) may contain aminoacids in addition to Oral Rehydration Salts conforming to the parameters specified above and labeled with the indication " For Adult Choletric Diarrhoea only." (d) Patent and Proprietary Oral Rehydration Salts shall not contain Mono or Polysaccharides or saccharine sweetening agent.
633(E)	13.09.1995	53. Fixed dose combination of Oxyphenbutazone or Phenylbutazone with any other drug. 54. Fixed dose combination of Analgin with any other drug. (the words 'other than anti-spasmodics' omitted by G.O.I. notification no.405(E) dt.03.06.1999.) 55. Fixed dose combination dextropropoxyphene with any other drug other than anti-spasmodics and/or non-steroidal anti-inflammatory drugs (NSAIDS). 56. Fixed dose combination of a drug, standards of which are specified in the Second Schedule to the said Act with an Ayurvedic, Siddha or Unani drug.
499(E)	14.08.1998	57. Mepacrine Hydrochloride (Quinacrine and its salts) in any dosage form for use for female sterilization or contraception. 58. Fenfluramine and Dexfenfluramine.
169(E)	12.03.2001	59. Fixed dose combination of diazepam and diphenhydramine Hydrochloride.
885(E)	11.12.2009	60. Rimonabant

G.S.R.	Date of notification	Drug
93(E)	25.02.1997	Fixed dose combination of Penicillin with Streptomycin w.e.f. 01.01.1998.
702(E)	14.10.1999	(ii) Fixed dose combination of Vitamin B1,Vitamin B6 and Vitamin B12- w.e.f. 01.01.2001
814(E)	16.12.1999	1. Fixed dose combination of haemoglobin in any form (natural or synthetic.) 2. Fixed dose combination of Pancreatin with Pancrelipase containing amylipase, protease and lipase wqith any other enzyme. w.e.f.01.09.2000
170(E)	12.03.2001	1. Fixed dose combination of Nitrofurantoin and trimethprim. 2. Fixed dose combination of Phenobarbitone with any anti-asthmatic drug. 3. Fixed dose combination of Phenobarbitone with Hyoscine and/or Hyoscyamine. 4. Fixed dose combination of Phenobarbitone with Ergotamine and or Belladonna. 5. Fixed dose combination of Haloperidol with any anti-cholinergic agent including Propanthelene Bromide. 6. Fixed dose combination of Nalidixic acid with any anti-amoebics including Metronidazole. 7. Fixed dose combination of Loperamide Hydrochloride with Furazolidone. 8. Fixed dose combination of Cyproheptadine with Lysine or Peptone. w.e.f. 01.01.2002
510(E)	25.07.2005	Valdecoxib and its formulations for human use.
499(E)	04.07.2008	Diclofenac and its formulations for animal use.
910(E)	12.11.2010	Rosiglitazone and its formulations for human use.
82(E)	10.02.2011	1. Nimesulide formulations for human use in children below 12 years of age. 2. Cisapride and its formulations for human use. 3. Phenylpropanolamine and its formulations for human use. 4. (replaced by G.S.R. 418(E) dt. 30.05.2011) 5. Sibutramine and its formulations for human use, and 6. R- Sibutramine and its formulations for human use.

G.S.R.	Date of notification	Drug
218(E)	16.03.2011	(i) Gatifloxacin formulation for systemic use in human by any route including oral and injectable; and (ii) Tegaserod and its formulations for human use.
418(E)	30.05.2011	Human placental extract and its formulations for human use except its (i) topical application for wound healing, and (ii) injection for pelvic inflammatory disease Subject to the condition that the manufacturers of Human placental extract and its formulations for human use shall maintain meticulous details and traceability of the donors of the placenta so as to ensure that the placenta is collected from the donors who are free from HIV, HBsAg, HCV and other viruses.
752(E)	12.10.2011	Letrozole for induction of ovulation in anovulatory infertility.
332(E)	23.05.2013	Dextropropoxyphene and formulations containing Dextropropoxyphene for human use.
377(E)	18.06.2013	Fixed dose combination of Flupenthixol + Melitracen for human use.
378(E)	18.06.2013	Analgin and all formulations containing Analgin for human use.
379(E)	18.06.2013	Pioglitazone and all formulations containing Pioglitazone for human use.

Pharmacists should not dispense any of these drugs and educate people not to consume these even if any old stock is left with them. Every pharmacist should keep track of any product banned in future and update his list for ready reference.

Rapid Determination & Standardization of Garcinia Fruit Extract of Hydroxycitric acid (HCA) in *Garcinia cambogia* by HPLC

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Abstract

A simple HPLC (high performance liquid chromatography) sensitive isocratic method for the detection and quantification of HCA in *Garcinia combogia* has been developed. HCA is major acid component was shown to be potent inhibitor of alpha amylase and alpha glucosidase leading to reduction of carbohydrate metabolism, results in weight loss. Content of HCA present in plant extract has been found to be in range of 50% using reversed-phase HPLC with ultraviolet detection. Separation was achieved on a C_{18} (Phenomenex 5 µm, 4.6 x 250 mm) reversed-phase column. The mobile phase consisting of 0.1M Na_2SO_4 aqueous solution, Ph adjusted to 2.5 with 20% v/v H_2SO_4, was delivered at a flow rate of 1.0 ml/min. Linearity was observed in the range of 20-100 ppm with correlation coefficient of 0.9997. Relative standard deviation of linearity of the method was found to be 1.27%. Detection limit was 5.0 ppm and quantification limit was 7.5 ppm. The repeatability of the method was found to be 0.69 RSD. Recovery values from 99.87-100.10% indicate best accuracy of the method. Methanolic extract of *Garcinia combogia* was analyzed by this method and found to contain 50-55% HCA. The proposed method was found to be precise and accurate for quantification of HCA in *Garcinia combogia*.

Keywords: *Garcinia combogia*, HCA, HPLC,

Introduction

Garcinia (*Garcinia cambogia*) is a diminutive purple fruit native to Sri Lanka and South-east Asia. It is used as a weight loss aid, but the evidence is inconclusive. The rind is rich in Hydroxycitric acid (HCA) and has been used for centuries throughout Southeast Asia as a food preservative, flavoring agent and carminative (induces expulsion of gas from stomach or intestines). According to Indian folk tradition, *Garcinia cambogia* is used for rheumatism and bowel complaints. Neither acute nor chronic toxicity is reported with regular consumption of Garcinia products as either food or tonics. These products have been used routinely in the coastal areas of South Asia for centuries and continue to be consumed in large amounts. There is preliminary evidence for the use of Garcinia in exercise performance and weight loss, although currently, available evidence is mixed. Among the components of the *Garcinia cambogia* extract, (-)-hydroxycitric acid exists as a free acid and as hydroxycitric acid lactone forms. The free acid form is considered to be biologically

active. However, the free acid is unstable and is converted to its more stable lactone form. For consumer products, the free acid is often stabilized by forming salts of (-)-Hydroxycitric acid (Rajpal *et al*., 2002). It also contains tartaric acid reducing sugars and phosphoric acid (as calcium triphosphate). It limits the synthesis of fatty acids in the muscles and liver lipogenesis (production of fat) by inhibiting the enzyme ATP-citrate lyase. Fruits contain 30% acid (calculated as Citric acid) essentially (-)-hydroxycitric acid. Several other compounds have also been identified, including Camboginol & Cambogin. The resin possesses purgative properties.

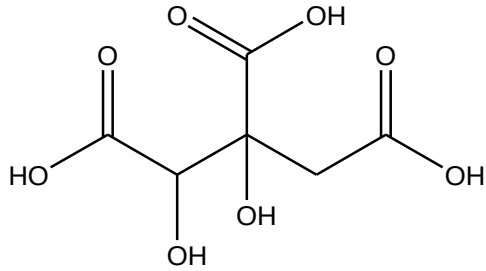


Figure 1: (-)-Hydroxycitric acid

Materials and Methods

Chemicals

All other reagents used were of HPLC grade or AR grade as per requirement.

Plant Materials

G. combogia plant procured from Sri Lanka, identified by Taxonomist. A voucher specimen has been maintained at R&D centre, Sanat Products Ltd., Sikandrabad, India. The active compound Hydroxycitric acid (HCA) was purchased from Natural Remedies Pvt. Ltd, Bangalore.

Extraction Method of Garcinia combogia

Raw material: The powdered sample of *Garcinia combogia* fruit passed through 20 mesh sieve, 5 gm of Garcinia powder was weighed. 50 ml of water was added and refluxed for 30 min at temperature 60^0-70^0C. Then, it was filtered. Again twice extract the material using water and filtered. All the extracts were combined and concentrated up to 100 ml.

Extraction Method of Garcinia extracts

The air dried powdered sample of *Garcinia cambogia* fruit was passed through 20 mesh and 100 gm of this powder was weighed and extracted three times with 400 ml of 85% Methanol for 3 hrs at 60^oC. Extract was filtered and the above process was repeated three times. All the extracts were combined and concentrated by distillation. Thick paste was attained, which was kept at room temperature for 24 hrs, filtered and refluxed with charcoal (10% of weight) for 1 hr. Further filtered through vacuum filter and kept at room temperature and after cooling, 10% of calcium salt

according to filtrate was added and stirred for 5-10 min. It was concentrated in Rota Vapour (Buchi). Pure Methanol was added to it and kept for precipitation for half an hour. Precipitate was separated, dried and weighed.

HPLC Assay

The HPLC system used for the analysis, was equipped with a Autosampler, UV-Detector, of model Shimadzu, LC 2010A, Japan, An RP C_{18} (Phenomenex, 5 µm, 4.6 x 250 mm) column. The mobile phase consisted of 0.1M Na_2SO_4 aqueous solution. The pH of the mobile phase was adjusted to 2.5 with 20% v/v Sulphuric acid (H_2SO_4), The mobile phase was filtered through 0.45 µm Millipore filter and degassed by sonication for 30 min and detection at the maximum UV absorption of 210 nm was performed. The flow rate was 1.0 ml/min. Injection volume was adjusted to 20 µl and detection was made at 210 nm.

Preparation of stock and calibration of standard solutions

Standard solution of pure HCA (Hydroxy citric acid) was prepared by dissolving 2.0 mg in 20 ml (100 ppm) of HPLC grade water in a volumetric flask (stock solution). For the determination of limit of detection (LOD) and limit of quantification (LOQ), 2 ml of the stock solution was diluted to 10 ml (20 ppm). 4 ml of the same stock solution was diluted to 10 ml (40 ppm) for linearity study.

Preparation of Sample Solution

Approx. 50 mg powder of sample was taken and dissolved in 15 ml HPLC grade water. The sample was sonicated for 20 min. After sonication, the volume was made up to 50 ml with HPLC grade water and filtered through 0.45 µm membrane filter.

Calibration Curve

A serial dilution was made for stock solution at concentration of 20, 40, 60, 80, 100 ppm. The linearity of the method for Standard was established by injection of different concentration in triplicate. Regression equation and co-efficient of correlation (r^2) was derived (Table 1).

Table 1: Validation parameters of the developed HPLC method for Quantification of HCA.

Validation Parameters	Results
Linearity range (ppm)	20-100 ppm
Correlation coefficient (r^2)	0.9997
Regression equation	y = 138.58x+1333.7
LOD (ppm)	5.0
LOQ (ppm)	7.5
Method precision (RSD %)	0.69
Intermediate precision (RSD %)	
Interday (%)	0.82-1.33

Validation Parameters	Results
Intraday (%)	1.16-1.25
RSD% (Linearity of method)	1.27

Validation of HPLC Method

Limits of Detection (LOD) and Quantification (LOQ)

For determination of the limit of detection (LOD) and limit of quantification (LOQ), different dilutions of the standard solution were analyzed 6 times using mobile phase as a blank. The LOD and LOQ were determined on the basis of signal-to- noise ratio until the average responses were approximately three and ten times the responses of the blank respectively.

Accuracy (recovery)

Accuracy of the method was ascertained by spiking the pre-analyzed samples with known amount of standard solution (50%, 100%, and 150%). The average percentage recovery was estimated by applying values of peak area to the regression equations of the calibration graph. Three replicate samples of each concentration level were prepared.

Method Precision (Repeatability)

The precision of the instruments was checked by repeatedly injecting and analyzing (n=6) standard solution 60 ppm. The results are reported in terms of relative standard deviation (RSD).

Intermediate Precision (Reproducibility)

The interday and intraday precision of the proposed method were determined by analyzing standard solution at different concentrations (20, 40, 60, 80, 100 ppm) three times on the same day and on three different days. The results are reported in terms of RSD.

Results and Discussion

Development of HPLC Method

The retention times for HCA was 3.399 min, respectively and baseline was relatively free from drift the proportions of the organic and aqueous phase were adjusted to obtain a rapid and simple assay method with reasonable run time, suitable retention time and sharp peak. Under optimized conditions using isocratic mixture of anhydrous sodium sulphate and water as mobile phase results in well resolved symmetric peak for HCA Study. The maximum absorbance was observed at 210 nm. Hence 210 nm was used for HPLC detection; one other peak was also observed which may be due to presence of some other acid present in raw material. Content of HCA in fruit extract was found to be 50-55%. The total run time of HCA was found to be 10 minutes and the HCA appeared on chromatogram at 3.396, 3.398 minutes in raw material (Figure 2B) and 50% extract (Figure 2C) respectively. The retention time of reference standard (HCA) was observed to be 3.399 minutes (Figure 2A). This indicates that the present HPLC method is rapid; easy and convenient. When the same drug solution was injected 6 times, the retention time of the peak was found to be same.

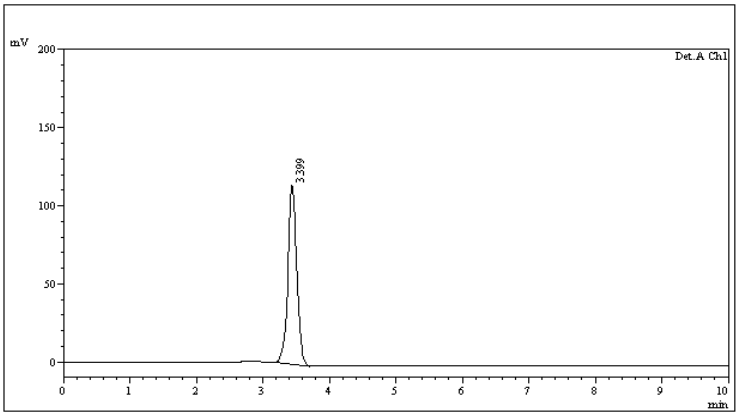


Figure 2 (A): HPLC chromatogram of Reference Standard (HCA)

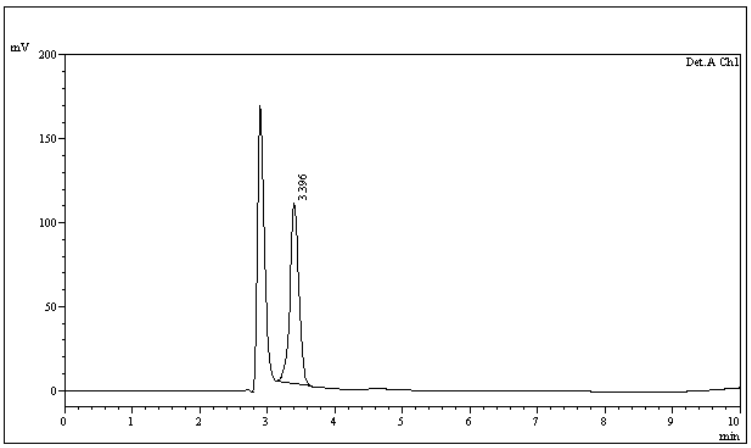
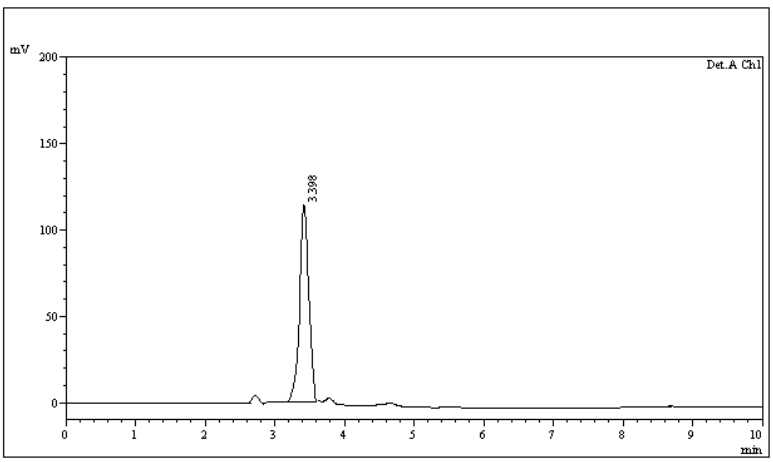


Figure 2 (B): HPLC chromatogram of raw material of *Garcinia combogia*



(C)

Figure 2 (C): HPLC chromatogram of *Garcinia combogia* extract

Method Validation

The calibration curve was prepared by plotting the peak area against standard concentration; it was found linear in the range of 20-100 ppm. The regression equation was found as y = 138.58x+1333.7 with r^2 of 0.9997, showing best linearity. The method was validated in terms of precision, repeatability, accuracy and other validation method parameters. The repeatability of the HPLC method and intermediate precisions for intra-day and interday variations are given in Table 1. The LOD value was found to be 5 ppm, which is the concentration that yields a signal-to-noise (S/N) ratio of 3:1. The LOQ value under the described conditions was 7.5 ppm with an S/N ratio of 10:1. This confirmed the sensitivity for quantification of compound. A recovery value from 99.87 to 100.10% indicates best accuracy of the method.

Conclusion

A method for analysis of *G. combogia* using HCA as analytical marker was developed. The method was found to be simple, precise, specific, sensitive and accurate. The present work is simple and sufficiently selective for the determination of HCA in commercial sample of *G. combogia.*

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Antidiabetic Activities of Selected Medicinal Plants and their Status in Indian Pharmacopoeia

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Abstract

The burden of diabetes is very high in India. India is one of the culturally diverse countries in the world where naturally derived medicines are used in preventing or treating illness. Several medicinal plants/medicinal herbs with hypoglycemic and other beneficial properties as reported in scientific literature have been known to control diabetes. Additionally, medicinal plants of natural origin have relatively less side effects. It has been reported that more than 550 herbal plants have been used for the management of diabetes. Twenty four medicinal plants with special reference to antidiabetic activity in animals models of diabetes, clinical studies wherever available and related beneficial or harmful effects along with family, common names, parts of the plant used, geographical source and chemical constituents have been described in this review. These include *Aegle marmelos, Allium sativum, Andrographis paniculata, Azadirachta indica, Casearia esculenta, Caesalpinia bonducella, Catharanthus roseus, Coccinia indica, Cuminum cyminum, Curcuma longa, Citrullus colocynthis, Enicostemma littorale, Emblica officinalis, Ficus bengalensis, Gymnema sylvestre, Helicterus isora, Momordica charantia, Phyllanthus niruri, Polyalthia longifolia, Pterocarpus marsupium, Salacia oblonga, Syzygium cumini, Tinospora cordifolia and Trigonella foenum graecum.* An attempt has also been made to find out their status in Indian Pharmacopoeia 2014.

Keywords: Medicinal plants, Antidiabetic activity, Indian Pharmacopoeia 2014

Introduction

In the last few years, herbal medicines have gained popularity because of their natural origin and less side effects. Around 80% of world population in developing countries relies on herbal medicines. About 21,000 plants have been listed by World Health Organization (WHO) which are used for medicinal purposes around the world, about 2500 of these listed plants are found in India. Plant based medicines have been used in India from very early times, as well as in China and other ancient cultures for the treatment of various ailments.[1] Folk medicine and traditional healing systems around the world have been using plant derivatives with hypoglycemic properties from old times.[2] It has been reported that 30% of patients with diabetes mellitus use complementary and alternative medicines.[3]

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion,

insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism[4]. Diabetes mellitus is of 4 types, of which two major types are - type 1 and type 2. The two main forms of type1 diabetes are type 1a which is thought to be due to immunological destruction of pancreatic ß cells resulting in insulin deficiency; and type 1b (idiopathic), in which there is no evidence of autoimmunity. Thus type 1 patients require insulin therapy for survival.[5] On the other hand, type 2 diabetes is the commonest form of diabetes and is characterized by disorders of insulin secretion and insulin resistance, however, type 2 diabetes may not require insulin[6.]

Diabetes mellitus is considered as one of the five leading causes of death in the world.[7] According to The International Diabetes Federation, by 2025 diabetic population will rise to 333 million amounting 6.3% of the world's population. India is considered as a host to the largest diabetes population in the world with an estimated 35 million people, amounting to 8% of the adults.

The current comprehensive review focuses on pharmacological studies of selected medicinal plants in experimental models of diabetes mellitus. The official status of these selected plants in IP is also enlisted.

STATUS OF THESE SELECTIVE MEDICINAL PLANTS IN INDIAN PHARMACOPOEIA

In India, Indian Pharmacopoeia (IP) is the legal and official book of standards of drugs and drug formulations as per the second schedule to the Drugs and Cosmetics Act 1940 and Rules 1945 thereunder. The standards prescribed in IP are set out after a thorough public comments and review process. The standards are authoritative and represent the minimum quality specifications with which an article must comply. The drug manufacturers must ensure that the article is manufactured in accordance with IP standards. If a drug monograph is not present in IP then other applicable suitable standards should be used.

To facilitate understanding and adoption of IP standards by stakeholders Guidance Manual for Compliance of IP has been brought out by the IP Commission in 2012. It addresses the "Guidelines for the quality of herbal drugs and its products" in addition to other content.

Special attention has been given to herbal monographs in IP. The status of these selected medicinal plants in IP 2014 is mentioned below.

Table 1: Status information of herbal drugs in Indian Pharmacopoeia 2014

S No.	Herb/Herbal Product	IP 2014
1	*Aegle marmelos*	**X**
2	*Allium sativum*	√
3	*Andrographis paniculata*	√
4	*Azadirachta indica*	√
5	*Casearia esculenta*	**X**

S No.	Herb/Herbal Product	IP 2014
6	*Caesalpinia bonducella*	**X**
7	*Catharanthus roseus*	**X**
8	*Coccinia indica*	**X**
9	*Cuminum cyminum*	√
10	*Curcuma longa*	√
11	*Citrullus colocynthis*	**X**
12	*Enicostemma littorale*	**X**
13	*Emblica officinalis*	√
14	*Ficus bengalensis*	**X**
15	*Gymnema sylvestre*	√
16	*Helicteres isora*	**X**
17	*Momordica charantia*	**X**
18	*Phyllanthus niruri*	√
19	*Polyalthia longifolia*	**X**
20	*Pterocarpus marsupium*	√
21	*Salacia oblonga*	**X**
22	*Syzygium cumini*	**X**
23	*Tinospora cordifolia*	√
24	*Trigonella foenum-graecum*	√

It is evident from the above table that 11 monographs are official in IP 2014.

The manufacturers must use IP for controlling the quality of manufactured drugs. If a drug monograph does not figure in IP, but is present in immediately preceding edition of IP, then the standards mentioned in that Pharmacopoeia apply. The standards prescribed in IP are to establish the compliance with regulatory requirements on an article. The interpretation of a monograph must be in accordance with all the general requirements, testing methods, text and notices pertaining to it in the IP. The manufacturers are advised to keep themselves abreast with IP, its Addendum, Amendment list and any other notice/ circular issued from IP Commission from time to time.

1. *Aegle marmelos*

Family: Rutaceae

Common name: Wood apple, Bael

Parts used: Fruits & Leaves

Geographical source: Throughout India, especially Central and Southern India, Burma/Myanmar.

Chemical constituents: Tannins, marmelosin, alkaloids (aegelin & aegelinin) and coumarins (marmesin, scopoletin), essential oils (α-phellandrene, limonene,β-ocimene, α-pinene,

caryphyllene, β-elemene, germacrene B)[8].

Pharmacological studies: The leaf extract of *Aegle marmelos* in streptozocin-induced diabetes has been reported to repair the injured pancreas in experimental rats.[9] The aqueous extract of seeds and leaves have shown hypoglycemic and antihyperglycemic activities in normal and diabetic rats as reported. [10,11] Administration of *A. marmelos* fruit extract at doses of 125 and 250 mg/kg twice daily to diabetic rats for a period of 30 days resulted in a significant increase in insulin levels associated with a significant decrease in fasting blood glucose levels.[12] Maity P *et al*, 2009 have reviewed the various biological activities of *A. marmelos*.[13] The fruit aqueous extract at doses of 250, 500 and 1000mg/kg provided protective effect in type 2 diabetic rats by preserving β-cell function and insulin sensitivity through increased peroxisome proliferator activated receptor-γ (PPAR γ) expression.[14]

2. *Allium sativum*

Family: Liliaceae.

Common Name: Lasun

Parts used: Ripe Bulbs.

Geographical source: Central Asia, Southern Europe, USA, India.

Chemical constituents: Allicin produced enzymatically from allin, carbohydrate, organosulphur compound such as proteins, free amino acid (mainly arginine), fibers, lipids, phytic acid, saponins.

Pharmacological studies: S-allyl cysteine sulphoxide (SACS), a sulphur containing amino acid of garlic which is a precursor of allicin and garlic oil, showed significant antidiabetic effects in alloxan-induced diabetic rats. Administration of S-allyl cysteine sulphoxide at a dose of 200 mg/kg body weight significantly decreased the concentration of serum lipids, blood glucose and activities of serum enzymes like alkaline phosphatase, acid phosphatase, lactate dehydrogenase and liver glucose-6-phosphatase. It also showed significantly increased liver and intestinal HMG-CoA reductase activity and liver hexokinase activity.[15] Oral administration of the garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) for 14 days significantly decreased serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, AST and ALT levels, while increased serum insulin in diabetic rats but not in normal rats.[16] It has been reported to interact with chlorpropamide, fluindione, ritonavir and warfarin; it also reduced plasma concentrations of chlorzoxazone.[17]

3. *Andrographis paniculata*

Family: Acanthaceae

Common name: Kalmegh

Parts used: Whole plant

Geographical source: India, Pakistan, Indonesia, extensively cultivated in China and Thailand, the East and West Indies and Mauritius

Chemical Constituents: Andrographolide and neoandrographolide.

Pharmacological studies: Oral administration of the *Andrographis paniculata* extract at different doses (0.1, 0.2, and 0.4 g/ kg of body weight) significantly reduced the fasting serum glucose level in STZ-diabetic rats.[18] In another study, oral administration of *A. Paniculata* aqueous leaf extract has shown antidiabetic activity against streptozotocin-induced diabetes in animals owing to its beneficial antioxidant properties.[19] Andrographolide, the primary active component of *A. paniculata*, lowered plasma glucose in STZ-diabetic rats by increasing glucose utilization.[20] The ethanolic extract of whole plant has been found to have antihyperglycemic property and reduces oxidative stress in diabetic rats.[21] In another model, Hypoglycemic effect of the purified extract and andrographolide is also reported in high-fructose-fat diet induced diabetics in rats .[22] The extract has been shown to accelerate the metabolism of tolbutamide in rats, however it was found not to impair the hypoglycemic activity of tolbutaimide.[23]

Glucose metabolism and diabetogenic gene expression analysis of chloroform fraction of *A. paniculata* whole herb in diabetic albino mice was performed. The screening at molecular level revealed significant antidiabetic activity.[24]

4. *Azadirachta indica*

Family: Meliaceae

Common Name: Neem

Parts used: Whole plant

Geographical source: It is a tree native to India, Burma, Bangladesh, Sri Lanka, Malaysia and Pakistan, growing in tropical and semi-tropical regions.

Chemical constituents: Nimbidin, nimbin, nimbinin, nimbidinin, nimbolide, nimbilic acid, gedunin, mahmoodin, azadirachtin, gallic acid, margolonon

Pharmacological studies: The leaf extract and seed oil of *A. indica* is reported to possess antidiabetic activity against alloxan-induced diabetes in rabbits.[25] The mechanism of action was invesigated and it was found that the aqueous extract of leaf (500 mg/kg body weight) and bark (100 mg/kg body weight) when given once daily for 21 days to separate groups of diabetic rats strengthened antioxidant defense in alloxan-diabetic rat tissues.[26]

In patients with type-2 diabetes mellitus taking oral hypoglycemic agents with history of inadequate control, subjects were given low (0.5 g three times a day) and high (2 g three times a day) doses of powdered part, aqueous extract and alcoholic extract of *A. indica* for 14 days. On 15th day blood and urine samples for glucose were taken. It was found that *Azadirachta indica* has significant hypoglycemic activity in high dose and can be successfully combined with oral hypoglycemic agents in type-2 diabetic patients whose diabetes is not controlled by these agents.[27] Ethanolic extract of *A. indica* stem barks may not be safe as an oral remedy most especially at 100, 200 and 300 mg/kg body weight. Alterations in biochemical parameters by the ethanolic extract of *A. indica* stem bark indicated adverse effects on the various organs of the animals.[28] Treatment with neem oil and azadirachtin in diabetic pregnant rats has shown negative effect on maternal outcome .[29]

5. *Casearia esculenta*

Family: Flacourtiaceae

Common Name: Carilla

Parts used: Roots

Geographical source: A shrub richly distributed in Konkan plateau and South India **Chemical constituents:** 3-hydroxymethyl xylitol

Pharmacological studies: Root extract of *Casearia esculenta* showed significant reduction in blood glucose level, a decrease in the activities of glucose-6-phosphatase and fructose-1,6-biphosphatase and an increase in the activity of liver hexokinase, indicating potent hypoglycemic activity. Further studies were undertaken to evaluate the antioxidant potential of *C. esculenta* in STZ diabetic rats. Oral administration of *C. esculenta* root extract at doses of 200 and 300 mg/kg body weight for 45 days resulted in significant reduction in plasma thiobarbituric acid reactive substances (TBARS), hydroperoxide and ceruloplasmin and a significant elevation in plasma reduced glutathione (GSH) was observed indicating antioxidant activity of *C. esculenta* root extract.[30]

6. *Caesalpinia bonducella*

Family: Leguminosae

Common name: Nicker tree

Parts used: Seeds and leaves

Geographical Source: It is found in tropical parts of Asia & Africa.

Chemical constituents: Bitter principle (bonducin), steroidal saponin, fatty acids, hydrocarbons, phytosterols, isoflavones, amino acids, and phenolics.

Pharmacological studies: The aqueous and 50% ethanolic extract of *C bonducella* seeds exhibited significant antihyperglycemic effect in streptozotocin-diabetic rats.[31] In another study, significant hypoglycemic effect of aqueous and ethanolic extract of *C. Bonducella* seeds was observed in type 2 diabetic model in Long-Evans rats.[32]

Potent hypoglycemic activity of aqueous and ethanolic extracts of *C. Bonducella* is also reported in chronic type 2 diabetic model in Long-Evans rats.[33]

7. *Catharanthus roseus*

Family: Apocynaceae

Common name: Madagascar periwinkle

Parts used: Leaves, Roots

Geographical Source: Grown on commercial scale in South Africa, India, Australia and America.

Chemical constituents: Ajmalicine, serpentine, vinblastine, vincristine catharanthine, vindoline

Pharmacological studies: Oral administration of leaf juice of *Catharanthus roseus* at doses of 0.5,

0.75 and 1.0 ml/kg body weight in both normal and alloxan-diabetic rabbits showed dose dependent reduction in blood glucose level and the results were comparable to that of glibenclamide. [34] In another study, dichloromethane: methanol extract (1:1) of leaves and twigs of *C. roseus* (500 mg/kg p.o., for 7 and 15 days) showed therapeutic and prophylactic activity in streptozotocin-induced diabetic rats. The authors have suggested probable mode of action of active compounds of *C. roseus* through enhanced secretion of insulin from β-cells of Langerhans or through extra pancreatic mechanism. The results indicated increased metabolism of glucose and antioxidant mechanism.[35]

The co-administration of methanolic extract of *C. roseus* significantly increased the hypoglycemic effect of metformin against alloxan-induced diabetes in rats.[36] Rasineni K *et al*, 2010 have also reported antihyperglycemic activity of *C. roseus* leaf powder in streptozotocin-induced diabetes in male wistar rats at an oral dose of 100mg/kg body weight /day for 60 days. The decreased levels of hepatic and muscle glycogen content and alteration in glucose metabolism enzymatic activities were prevented with *C roseus* administration.[37]

8. *Coccinia indica*

Family: Cucurbitaceae.

Common name: Ivy gourd

Parts used: Leaves.

Geographical Source: Creeper which grows wild in Bangladesh and in many parts of the Indian sub-continent.

Chemical constituents: Heptacosane, Cephalandrol, triacontane, β-sitosterol, alkaloids cephalandrine A and cephalandrine B, aspartic acid, glutamic acid, asparagine, tyrosine, histidine, phenylalanine, threonine, valine and arginine.

Pharmacological studies: Oral administration of ethanolic extract of *C.indica* leaves (200mg/kg body weight) for 45 days has shown protective effect on changes in the fatty acid composition in streptozotocin-induced diabetic rats. The effect was found to be better than glibenclamide. [38]

Clinical investigation of dried extracts of *C. indica* (500 mg/kg body weight) to diabetic patients for 6 weeks corrected the elevated enzymes G-6-pase, LDH in glycolytic pathway and restored the LPL activity in lypolytic pathway with the control of hyperglycemia in diabetes.[39] A double blind control trial with preparation from the leaves of the plant on uncontrolled, maturity onset diabetics was carried out that lasted for six months. The patients who received the experimental preparations showed marked improvement in their glucose tolerance.[40]

9. *Cuminum cyminum*

Family: Apiaceae

Common Name: Cumin or Jeera

Parts used: Seeds

Geographical source: East Mediterranean to East India

Chemical constituents: Essential oil **(c**uminaldheyde, γ-terpinene) [41] petroselinic acid, palmitic, linoleic acids.

Pharmacological studies: The methanolic seed extracts of *Cuminum cyminum* showed significant reduction in blood glucose, glycosylated hemoglobin, creatinine, blood urea nitrogen and improved serum insulin and glycogen (liver and skeletal muscle) content in streptozotocin induced diabetic rats. It also showed significant effects in controlling oxidative stress and inhibiting the advanced glycosylation end products (AGE) formation, which are implicated in the pathogenesis of diabetic microvascular complications. Cuminaldehyde has been reported to inhibit aldose reductase and alpha-glucosidase isolated from rat.[42]

10. *Citrullus colocynthis*

Family: Cucurbitaceae

Common name: Bitter apple

Parts used: Fruit

Geographical Source: North West, Punjab, Sindh, Central and Southern India, and Coromandal coast.

Chemical constituents: Colocynthin (bitter principle), colocynthein (resin), colocynthetin, pectin, fixed oil.

Pharmacological studies: Sebbagh N *et al,* 2009 have reported hypoglycemic activity of *C. colocynthis* oil against streptozotocin-induced diabetes in rats.[43] In another study, the aqueous extract has shown hypoglycemic activity against alloxan-induced diabetes in rats [44]

A two month clinical trial was conducted in 50 patients of type II diabetes to determine efficacy and toxicity of *C.colocynthis*. The data showed a significant decrease in HbA1c and fasting blood glucose levels. *C. colocynthis* fruit treatment improved glycemic profile without severe adverse effects in type II diabetic patients.[45]

11. *Curcuma longa*

Family: Zingiberaceae

Common Name: Turmeric

Parts used: Rhizome

Geographical Source: India, and Southeast asia

Chemical constituents: Curcuminoids (curcumin I is the main constituent), tumerone, atlantone, zingiberone.

Pharmacological studies: Streptozotocin-induced diabetic rats were treated with curcumin (15 and 30 mg/kg, p.o.) for 2 weeks. Chronic treatment with curcumin significantly attenuated both renal dysfunction and oxidative stress in diabetic rats. These results confirmed that curcumin, the active principle of turmeric (*Curcuma longa*), ameliorates diabetic nephropathy in rats.[46] The role of

curcumin in diabetes is presented in a systematic review by Zhang *et al*, 2013.[47]

12. *Enicostemma littorale*

Family: Gentiaceae

Common Name: Majmakbooti

Parts used: Whole plant

Geographical Source: Widely distributed in South America, Africa, and Asia.

Chemical constituents: Bitter principle (swertimarine) two alkaloids (one gentianine and other's name not confirmed), ophelic acid and tannins. Betulin, a triterpene sapogenin and C-glucoside named as verticilliside

Pharmacological studies: Oral administration of aqueous *E. littorale* whole plant extract (1 and 2 g/kg) to alloxan-induced diabetic rats daily for 45 days significantly decreased blood glucose, TBARS and increased GSH, SOD and catalase.[48] Methanol extract administration to diabetic rats for 20 days reduced blood glucose levels and also raised the serum insulin levels. The antioxidants status of the rats also improved.[49]

13. *Emblica officinalis*

Family: Euphorbiaceae

Common name: Amla or Indian gooseberry

Parts used: Dried fruits, fresh fruit, seed, leaves, rootbark, flowers

Geographical source: It grows in tropical and subtropical regions including Pakistan, Uzbekistan, Sri Lanka, South East Asia, China and Malaysia.

Chemical constituents: Vitamin C, tannins, ellagic acid, gallic acid, lupeol, leucodelphinidin, quercetin, rutin, corylagin, emblicanin A and emblicanin B, pedunculagin and punigluconin.

Pharmacological studies: Oral administration of 75% methanolic extract (100 mg/kg body weight) of *E. officinalis* fruit to normal and alloxan induced diabetic rats, resulted in significant lowering of serum glucose level in 4 hours. In another investigation on ethyl acetate extract of the fruit (20 and 40mg/ kg/day) when given orally for 20 days to streptozotocin diabetic rats, significantly improved glucose metabolism.The hydro methanolic extract of leaves of *E.officinalis* showed antidiabetic effect in streptozotocin-induced type 2 diabetes mellitus in rats.[50]

E. officinalis fruit was evaluated for anti-hyperglycaemic and lipid-lowering properties in human volunteers. The results indicated a significant decrease ($P < 0.05$) in fasting and 2 h post-prandial blood glucose levels on the 21st day in both normal and diabetic subjects receiving 1, 2 or 3 g *E. officinalis* powder per day as compared with their baseline values.[51]

14. *Ficus bengalensis*

Family: Moraceae

Common Name: Banyan tree

Parts used: Bark, Leaves and Fruits

Geographical Distribution: Asia, from India through Myanmar (Burma), Thailand, Southeast Asia, Southern China, and Malaysia.

Chemical constituents: 20-tetratriacontene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, β-sitosterol glucoside, *meso*-inositol, catechin, quercetin, genistein.

Pharmacological studies: The aqueous extract at a dose of 500mg/kg/day exhibited significant antidiabetic activity in normal and streptozotocin induced diabetic rats.[52] Treatment of diabetic rats for one month orally with the active principle leucocyanidin derivative isolated from the bark of *Ficus bengalensis*, at a dosage of 100 mg/kg, showed significant decrease in blood and urine sugar, certain lipid components in serum and tissues and glucose-6-phosphatase activity in liver, but significant increase in body weight and the activities of hexokinase and HMGCOA reductase in tissues as compared to diabetic control. The mechanism of action of the principle may be related to its protective/inhibitory action against the insulin degradative processes.[53]

15. *Gymnema sylvestre*

Family: Asclepiadaceae

Common Name: Gurmar, Gudmor

Parts used: Leaves, Bark

Geographical source: Asia, Topical Africa, Malaysia and Sri Lanka

Chemical constituents: Gymnemic acids (saponins) which include gymnemic acids I-VII, gymnemosides A-F, flavones, anthraquinones, pentatriacontane, hentriacontane, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin and its related glycosides and stigmasterol.

Pharmacological Studies: Water-soluble acidic fraction of the *Gymnema sylvestre* was tested in streptozotocin-induced diabetic rats where *G.sylvetre* raised the levels of insulin.[54] Clinically, an extract from the leaves of *Gymnema sylvestre* 400 mg/day was administered for 18-20 months as a supplement to the conventional oral drugs. During extract supplementation, the non-insulin-dependent diabetes mellitus patients showed a significant reduction in blood glucose, glycosylated haemoglobin and glycosylated plasma proteins, and conventional drug dosage could be decreased**.**[55]

16. *Helicterus isora*

Family: Sterculiaceae.

Parts used: Roots.

Common name: East-Indian screw tree

Geographical source: India, Pakistan, Nepal, Sri Lanka, Burma, Thailand, Malaysia, China, Australia

Chemical constituents: Betulic acid, daucosterol, sitosterol, cucurbitacin-β and

isocucurbitacin-β.

Pharmacological studies: Ethanolic extract of *H. isora* root showed significant reduction in plasma glucose, triglyceride and insulin levels at 300 mg/kg dose after 9 days of administration to insulin resistant and diabetic C57BL/KsJdb/db mice.[56] In another study, hot water extracts were analysed for glucose uptake activity and found to be significantly active at 200 mg/ml dose comparable with insulin and metformin.[74]

17. *Momordica charantia*

Family: Cucurbitaceae.

Common Name: Bitter gourd

Parts used: Fruits

Geographical source: India, China.

Chemical Constituents: Charantine, goyaglycoside, mormodicoside, 3β,25-dihydroxy-5β,19-epoxycucurbita-6,(23E)-diene, momordicine, karavilagenin, karavilagenin C, karaviloside karaviloside, 3,7,23-trihydroxy-cucurbita-5,24-diene-19-al , 3,7,25-trihydroxy-cucurbita-5,23-diene 19-al, 3,7-dihydroxy-25-methoxycucurbita-5,23-diene-19-al, kuguacin kuguacin A, kuguacin B, kuguacin E

Pharmacological Studies: Alcoholic extract of fruit pulp has been demonstrated to possess hypoglycemic action in normal glucose-primed rat model at 500 mg/kg dose level. It showed 25-30% the activity of tolubuatmide 100 mg/kg. In other investigations, unripe fruit juice, aqueous extract of fruit powder and acetone extract of fruit powder exhibited antihyperglycemic activity in different animal models of diabetes.[57]

The beneficial effects and mechanism of action of *M charantia* in the treatment of diabetes mellitus have been reviewed by Garau *et al*, 2003[58]. A few clinical studies are also reported.[59]

18. *Phyllanthus niruri*

Family: Euphorbiaceae

Common Name: Bhumyamalaki

Parts used: Entire plant

Geographical source: Throughout tropical and sub-tropical regions of Asia, America, and China.

Chemical Constituents: Alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, gallic acid, ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, lupeols, methyl salicylate, niranthin, nirtetralin, niruretin, nirurin, nirurine, niruriside, norsecurinines, phyllanthin, hypophylanthin, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal and tricontanol

Pharmacological studies: Chronic oral administration of methanol extract caused a significant dose-related reduction in blood glucose levels as well as total cholesterol and triglycerides levels in

diabetic and normoglycaemic rats.[60] Oral administration of ethanolic leaf extract (400 mg/kg body weight) for 45 days resulted in a significant (P<0.05) decline in blood glucose from 310.20. to 141.0 mg/dl and significant recovery in body weight of diabetic mice. There was also a significant (P<0.05) reduction in the activities of glucose-6-phosphatase and fructose-1-6-disphosphatase in liver, furthermore there was significant (P<0.05) increase in the activity of glucokinase in liver of diabetic mice when compared with that of diabetic control.[61]

19. *Polyalthia longifolia*

Family: Annonaceae.

Common Name: Indian mast tree

Geographical Distribution: Southern India, Sri Lanka, Southeast Asia

Parts used: Bark

Chemical constituents: Alkaloids, glycosides, saponins, polyphenolic compounds, diterpenoids and tannins.

Pharmacological studies: The chloroform extract of stem bark of *Polyalthia longifolia* was evaluated for its antidiabetic activity in alloxan-induced diabetic rats and euglycaemic rats after a single dose of 200 mg/kg p.o and prolonged treatment of 100 mg/kg p.o for 10 days. The results revealed significant antihyperglycemic activity.[62]

20. *Pterocarpus marsupium*

Family: Fabaceae

Common Name: Indian Kino Tree

Parts used: Bark & Heartwood

Geographical Distribution: Hilly regions throughout the Deccan Peninsula

Chemical Constituents: Pterostilbene, marsupin, epicatechin, liquiritigenin , isoliquiritigenin.

Pharmacological studies: Marsupin, pterosupin and Iiquiritigenin obtained from this plant showed antihyperlipidemic activity.[63] An aqueous extract of *Pterocarpus marsupium* wood, at an oral dose of 250 mg/kg, showed statistically significant hypoglycemic activity.[64] Aqueous extract of *Pterocarpus marsupium* at both doses of 100 and 200 mg/kg, decreased the fasting and postprandial blood glucose in type 2 diabetic rats. The aqueous extract at both doses significantly decreased the elevated TNF-α level in type 2 diabetic rats.[65]

21. *Salacia oblonga*

Family: Hippocrateaceae.

Common Name: Saptachakra, saptarangi

Parts used: Roots

Geographical Distribution: India, Sri Lanka and other Southeast Asian countries

Chemical Constituents: Flavonoids (Salacinol and kotalanol)

Pharmacological studies: The chloroform eluted fraction of the petroleum ether extract of the root bark of Salacia demonstrated potent hypoglycemic activity in rats when compared to tolbutamide.[66]

Effect of hydroalcoholic root extract of *Salacia oblonga* on the random blood glucose (RBG) levels, serum insulin, glycated haemoglobin (HbA1c) and the serum lipid profile in long standing, experimentally-induced diabetes mellitus in rats was studied using glibenclamide (Glb) as the standard. It improved the glycaemic parameters in diabetic rats after a prolonged treatment.[67]

22. *Syzygium cumini*

Family: Myrtaceae

Common Name:Jambul

Parts used: Fruits, Seeds, Stem Bark

Geographical Source: India, Bangladesh, Burma, Nepal, Pakistan, Sri Lanka, Indonesia

Chemical constituents: Anthocyanins, glucosides, ellagic acid, isoquercetin, kaemferol, myrecetin.

Pharmacological studies: Oral administration of 2.5 and 5.0 g/kg body weight of the aqueous extract of the seeds for 6 weeks resulted in significant reduction in blood glucose and an increase in total haemoglobin.The aqueous extract also decreased free radical formation which clearly showed the antioxidant property.[68]

23. *Tinospora cordifolia*

Family: Menispermaceae

Common Name: Gaduchi

Parts used: Roots

Geographical Source: Found in tropical areas of India

Chemical constituents: Alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides. Leaves of this plant are rich in protein (11.2%) and are fairly rich in calcium and phosphorus**.**

Pharmacological studies: Oral administration of an aqueous *T. cordifolia* root extract to alloxan-diabetic rats caused a significant reduction in blood glucose and brain lipids. Though the aqueous extract at a dose of 400 mg/kg could elicit significant antihyperglycemic effect in different animal models, its effect was found to be equivalent to only one unit/kg of insulin.[69]

24. *Trigonella foenum graecum*

Family: Papilionaceae

Common Name: Fenugreek

Parts used: Leaves & Seeds

Geographical Source: India and Northern Europe

Chemical constituents: Fenugreekine, trigonelline, trigonellic acid, lecithin

Pharmacological studies: Administration of 2 and 8 g/kg of plant extract produced dose dependent decrease in the blood glucose levels in both normal as well as diabetic rats.[70] Administration of fenugreek seeds improved glucose metabolism and normalized creatinine kinase activity in heart, skeletal muscle and liver of diabetic rats. It also reduced hepatic and renal glucose-6-phosphalase and fructose-1, 6-biphosphatase activity.[71] In another study, streptozotocin-induced diabetic rats were administrated by oral intragastric intubation separately with low dose (0.44 g/kg/day), middle dose (0.87 g/kg/day), high dose (1.74 g/kg/day) of *Trigonella foenum-graecum* extract, and metformin (0.175 g/kg/day) for 6 weeks. *Trigonella foenum-graecum* extract had decreased blood glucose, glycated hemoglobin, triglycerides, total cholestrol and higher high-density-lipoprotein-cholesterol in a dose-dependent manner. High and middle doses of *Trigonella foenum-graecum* extract showed significant reduction in the plasma viscosity, whole blood viscosity of high shear rate (200 s-1) and low shear rate (40 s-1), erythrocyte sedimentation rate, whole blood reduction viscosity in diabetic rats.[72] A double blind placebo controlled study to evaluate the effects of *Trigonella foenum-graecum* concluded adjunct use of fenugreek seeds improving glycemic control and decreasing insulin resistance in mild type-2 diabetic patients.[73]

Conclusion

An attempt has been made in this review to provide scientific literature of validated claims of selected medicinal plants with special reference to their antidiabetic activity and their official status in IP. We hope that this information will be useful to healthcare professionals, scientists and research scholars to carry out further studies for development of herbal medicines.

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Method Development, Validation and Stability Study of Perindopril in Bulk and Pharmaceutical Dosage Form by UV-Spectrophotometric Method

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Abstract

A simple, specific and reliable UV-VIS spectrophotometric method was developed for the estimation of Perindopril in bulk and pharmaceutical dosage forms. Water: methanol (8:2) was chosen as the solvent system. The λ_{max} was found to be 206nm and the responses were linear in the range of 10-100µg/ml. The regression equation of the calibration graph and correlation coefficient were found to be y = 0.018x + 0.088 and 0.992 respectively. The %RSD values for both intraday and interday precision were less than 1%. The recovery of the drug from the sample was ranged between 98.94% and 100.14%. The proposed method was validated for accuracy, precision, robustness, ruggedness, LOD and LOQ. While estimating the commercial formulation there was no interference of excipients and other additives. Hence this method can be used for routine determination of Perindopril in bulk and their pharmaceutical dosage forms. The proposed method for stability study shows that there was appreciable degradation found in stress condition of Perindopril.

Keywords: Perindopril, UV-VIS Spectrophotometric, Stability study, Degradation.

Introduction

Perindopril is a long-acting ACE inhibitor. It is used to treat high blood pressure, heart failure or stable coronary artery disease[1] in form of perindopril arginine (trade names include Coversyl, Coversum) or perindopril erbumine (trade name Aceon). According to the Australian government's Pharmaceutical Benefits Scheme website, based on data provided to the Australian Department of Health and Aging by the manufacturer, *perindopril arginine* and *perindopril erbumine* are therapeutically equivalent and may be interchanged without differences in clinical effect.[2] The *Anglo-Scandinavian Cardiac Outcomes Trial* showed the benefits of taking the two drugs Perindopril and Amlodipine together. The 9000 British patients aged 40 to 79 were involved in the five-year trial. Half were given the new drug combination, the rest were given traditional drugs. Perindopril and Amlodipine were found to be so effective that the trial was stopped early so that all patients could receive the combination.[3][4] UV Spectrophotometric method was developed and validated as per ICH guidelines.[5] Spectrophotometric method is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is

based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380 nm). [6-8]

Materials and Methods

Materials

The gift sample of Perindopril (Figure 1 & Table 1) was obtained from Glenmark Pvt. Ltd. The required analytical grade solvents were purchased from Sigma Aldrich Pvt. Ltd. UV-1800 model was used and the software was UV-probe 2.1.

Table 1: Some important properties of Perindopril

PROPERTIES	SPECIFICATIONS
Formula	$C_{19}H_{32}N_2O_5$
Form	Powder
Color	White
Molecular mass	368.46
Melting point	126-128^0 C
Solubility in water	Soluble in water
Storage temperature	2-8 °C

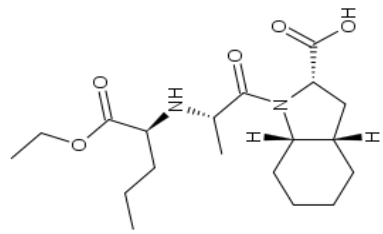

Figure 1: Structure of Perindopril

Methods

Determination of Working Wave Length

In order to determine the wave length of maximum absorption (λ_{max}) of the drug, different concentrations of Perindopril (10-100µg/ml) in water:methanol (8:2) were scanned using UV-VIS spectrophotometer within the wave length region of 200-400 nm against water: methanol (8:2) as blank. The resulting spectra were shown in Figure 2 and the absorption curve showed characteristic absorption maxima at 206 nm for Perindopril.

Preparation of Calibration Curve

For preparation of calibration curve of Perindopril, a stock solution of 1000µg/ml was prepared. From it different concentrations ranging from 10-100µg/ml prepared and were scanned at 206 nm in UV-VIS spectrophotometer. Then the respective absorbances were noted and the calibration curve was plotted by taking concentration on X- axis and absorbance on Y-axis. From the calibration curve it was found that it shows linearity in the range of 10-100µg/ml with regression coefficient 0.992. The calibration curve of Perindopril was shown in Figure 3.

Optical characteristics of Perindopril

The optical characteristics like Beer's Law Limit, Sandell's Sensitivity, Standard Deviation, % Relative Standard Deviation, Correlation Coefficient, Regression equation, Slope, Intercept and Absorption Maxima were determined and was given in Table 2.

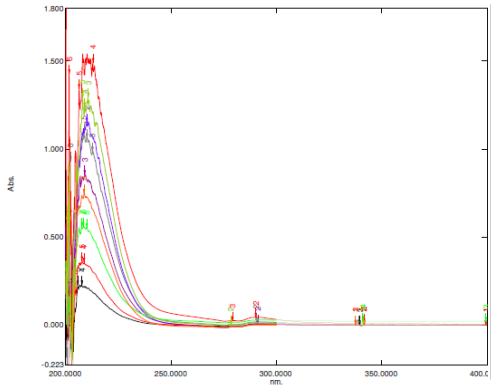

Figure 2: Overlay UV-VIS Spectra of Perindopril

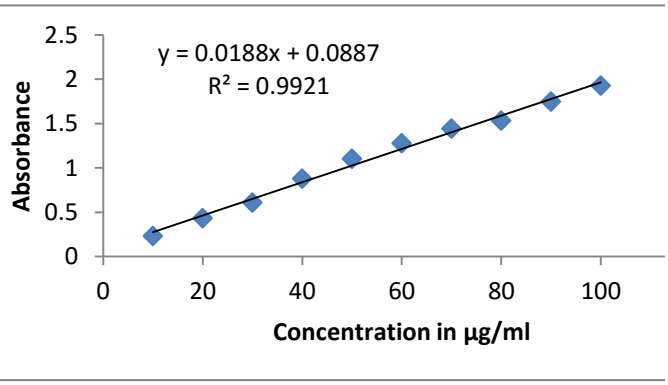

Figure 3: Calibration Curve of Perindopril

Table 2: Optical Characteristics of Perindopril

Beer's Law Limit	10-100 µg/ml
Sandell's Sensitivity (µg/cm2/0.001absorbance unit)	0.04273
Standard Deviation	0.006
% Relative Standard Deviation	0.0069
Correlation Coefficient	992
Regression equation (Y)	Y=0.018X+0.088
Slope(a)	0.018
Intercept(b)	0.088
Absorption Maxima	206

Validation parameters

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy data are given in Table 3.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. The precision data are given in Table 4. The intra-day precision data are given in Table 5. The inter-day precision data are given in Table 6.

Table 3: Accuracy Data of the UV-VIS Spetrophotometric Method for Perindopril

Samples	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Pure	Formulation		
S_1:80%	24	30	99.14	Mean = 98.94
S_2:80%	24	30	98.63	S.D = 0.281
S_3:80%	24	30	99.05	% R.S.D = 0.460
S_4:100%	30	30	100.11	Mean = 100.14
S_5:100%	30	30	100.25	S.D = 0.107
S_6:100%	30	30	100.07	% R.S.D = 0.491
S_7:120%	24	20	100.43	Mean = 99.68
S_8:120%	24	20	99.26	S.D = 0.895
S_9:120%	24	20	99.37	% R.S.D = 0.885

Table 4: Precision Data Showing Repeatability of the UV-VIS Spetrophotometric Method for Perindopril

Sl.No.	Concentration (µg/ml)	Abs.	Calculated amount (µg/ml)	Statistical Analysis
1	30	0.611	29.05	
2	30	0.617	29.38	Mean=29.37
3	30	0.608	28.88	S.D=0.205
4	30	0.622	29.66	%R.S.D=0.328
5	30	0.619	29.50	
6	30	0.615	29.27	

Table 5: Intra Day Precision Data of the UV-VIS Spetrophotometric Method for Perindopril

Conc.(µg/ml)	Abs.1	Abs.2	Abs.3	Statistical Analysis
30	0.631	0.623	0.595	
30	0.612	0.637	0.598	
30	0.625	0.633	0.593	Mean=29.27
30	0.609	0.620	0.603	S.D=0.312
30	0.635	0.613	0.607	%R.S.D=0.266
30	0.614	0.617	0.612	
Mean	0.621	0.623	0.601	
Calc.Amt. (µg/ml)	29.61	29.72	28.50	

Table 6: Inter Day Precision Data of the UV-VIS Spetrophotometric Method for Perindopril

Conc.(µg/ml)	Abs.(Day 1)	Abs.(Day 2)	Abs.(Day 3)	Statistical Analysis
30	0.641	0.607	0.591	
30	0.632	0.619	0.597	
30	0.617	0.601	0.590	Mean =29.53
30	0.640	0.592	0.603	
30	0.638	0.623	0.607	S.D =0.426
30	0.645	0.629	0.593	
Mean	0.635	0.611	0.596	%R.S.D =0.402
Calc. Amt. (µg/ml)	30.38	30.00	28.22	

Robustness/ Ruggedness

The definition for robustness/ruggedness applied is the robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained results, and a robustness test as an experimental set-up to evaluate the robustness of a method. The term ruggedness is frequently used as a synonym. Several definitions for robustness or ruggedness exist which are, however, all closely related. Robustness/ Ruggedness data are given in Table 7 and Table 8.

Table 7: Ruggedness Data of the UV-VIS Spectrophotometric Method by Different Analyst for Perindopril

Analyst-1				Analyst-2			
Conc. (µg/ml)	Abs.	Calc. Amt. (µg/ml)	Statistical Analysis	Conc. (µg/ml)	Abs.	Calc. Amt. (µg/ml)	Statistical Analysis
30	0.613	29.16	Mean=29.82	30	0.622	29.66	
30	0.625	29.83		30	0.637	30.50	Mean=30.09
30	0.627	29.94	S.D=0.713	30	0.618	29.44	S.D=0.081
30	0.642	30.77		30	0.644	30.88	%R.S.D=0.077
30	0.619	29.50	%R.S.D=0.641	30	0.627	29.94	
30	0.623	29.72		30	0.631	30.16	

Table 8: Robustness Data of the UV-VIS Spectrophotometric Method by Different Solvent Composition for Perindopril

Water: HCl (92:08)				Water: HCl (88:12)			
Conc. (µg/ml)	Abs.	Calc. Amt. (µg/m)	Statistical Analysis	Conc. (µg/ml)	Abs.	Calc. Amt. (µg/m)	Statistical Analysis
30	0.603	28.61		30	0.607	28.83	
30	0.628	30.00	Mean=29.32	30	0.600	28.44	Mean=29.30
30	0.617	29.38	S.D=0.085	30	0.613	29.16	S.D=0.277
30	0.633	30.27	%R.S.D=0.087	30	0.639	30.61	%R.S.D=0.218
30	0.609	28.94		30	0.615	29.27	
30	0.605	28.72		30	0.619	29.50	

Limit of Detection and Limit of Quantitation

The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determination of detection limit and quantification limits.

Detection limit= 3.3σ/s

Quantification limit= 10 σ/s

Where σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

The limit of detection (LOD) and limit of quantification (LOQ) data are given in Table 9.

Table 9: Limit of detection and Limit of quantitation of Perindopril

Sl.No.	Parameters	S.D	Slope(b)	Formula	Calculation(µg/ml)
1	LOD	0.006	0.018	$3.3(S.D/b)$	1.100
2	LOQ	0.006	0.018	$10(S.D/b)$	3.330

Assay and control of impurities

Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution). Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test. The assay data are given in Table 10.

Table 10: Assay Data of Perindopril Formulations

Formulation	Labeled claim (mg/tab.)	Observed Amount (±S.D) mg	% Recovery	%R.S.D
Perigard	02	1.96 ± 0.073	98.00	0.518
Coversyl	02	1.93 ± 0.069	96.25	0.913

Stability studies

Hydrolytic degradation

Hydrolytic degradation usually means the cleavage of chemical bonds by the addition of water. Generally, hydrolytic degradation or saccharification is a step in the degradation of a substance. This can be performed in three conditions that are neutral medium, acidic medium and basic medium.

Samples were withdrawn according to protocol. From the drawn sample solutions were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 5 hr at 60^0C. The neutral degradation data are given in Table 11 and UV spectrum are shown in Figure 4. The acidic degradation data are given in Table 12 and UV spectrum are shown in Figure 5. The basic degradation data are given in Table 13 and the UV spectrum are shown in Figure 6.

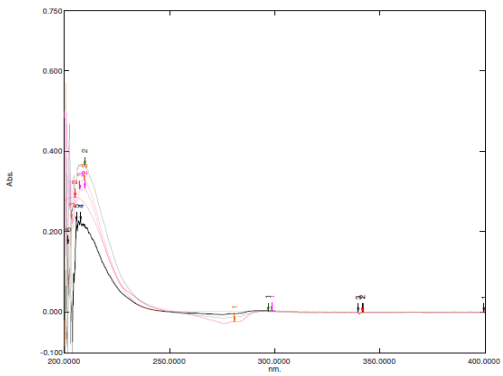

Figure 4: UV-VIS spectrum of Perindopril in Neutral Degradation

Table 11: Hydrolytic Degradation of Perindopril in Neutral Condition:

Sl.No.	Name	Abs.	Conc.	%Degradation
1	Drug	0.358	15	0
2	Degradation1	0.351	14.61	2.6
3	Degradation2	0.320	12.88	14.13
4	Degradation3	0.311	12.38	17.46
5	Degradation4	0.285	10.94	27.06

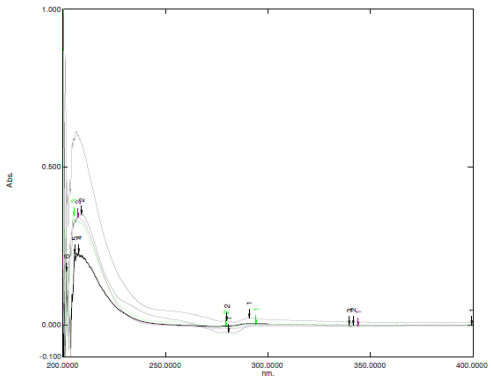

Figure 5: UV-VIS spectrum of Perindopril in Acidic Degradation

Table 12: Hydrolytic Degradation of Perindopril in Acidic Condition

Sl.No.	Name	Abs.	Conc.	%Degradation
1	Drug	0.628	30	0
2	Degradation1	0.468	21.11	29.63
3	Degradation2	0.453	20.27	32.43
4	Degradation3	0.417	18.27	39.10

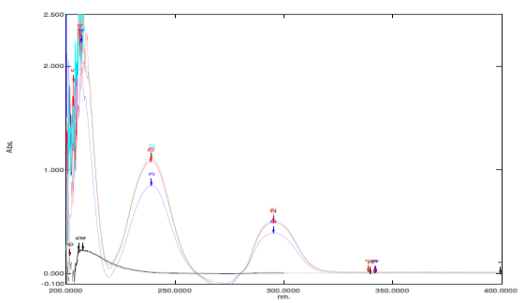

Figure 6: UV-VIS spectrum of Perindopril in Basic Degradation

Table 13: Hydrolytic Degradation of Perindopril in Basic Condition

Sl.No.	Name	Abs.	Conc.	%Degradation
1	Drug	1.888	100	0
2	Degradation1	1.716	90.44	9.56
3	Degradation2	1.635	85.94	14.06
4	Degradation3	0.539	25.05	74.95

Oxidative Degradation of Perindopril

Samples were withdrawn according to protocol. From the drawn samples 50 µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 5 hr at 60 °C. The required data are given in Table 14 and the UV spectrum is shown in Figure 7.

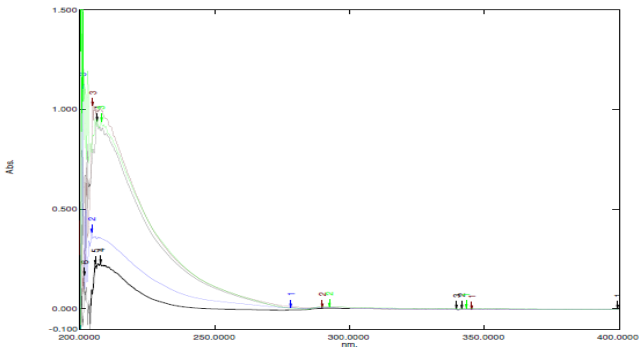

Figure 7: UV-VIS spectrum of Perindopril in Oxidative Degradation

Table 14: Oxidative Degradation of Perindopril:

Sl.No.	Name	Abs.	Conc.	%Degradation
1	Drug	0.988	50	0
2	Degradation1	0.872	43.55	12.90
3	Degradation2	0.459	20.61	58.78
4	Degradation3	0.407	17.72	64.56

Results and Discussion

The objective of the present work was development and validation of UV spectral study and degradation of Perindopril using UV spectrophotometer. The UV Spectra for Perindopril were recorded at the wavelength of 206 nm (λ_{max}). The method was found to be simple and the accuracy, precision, intra-day precision, inter-day precision, repeatability and assay were performed and the results were tabulated below. Appreciable degradation was found in Perindopril which may be taken into consideration during manufacture and storage of the drug.

Conclusion

The UV spectroscopic method has been successfully developed for Perindopril in the present work, which can be routinely employed in pharmaceutical Industries and Analytical Laboratories for their estimation in bulk and formulations.

Acknowledgement

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