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From The Chief Editor

Dear friends we are printing and publishing both journals for two decades and providing in time schedule but in 2020 we could not print and circulate both journals in time due to COVID-19 pandemic. Anyhow facing unfavorable conditions we have printed and published all three issues of both journals in one binding .Once again we all are facing worst condition of pandemic, however we are printing and publishing April and August 2021 issues of both journals together due to under lockdown conditions and sending you. December 2021 issues are in your hands, well in time. Considering present scenario we have decided to publish two issues of both journals in June and December 2022. We hope you will support and appreciate us for this decision during COVID-19 pandemic.

Dr. Ram Prakash

Prof. Barry K Sharpless
Noble Prize in Chemistry 2001

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FINGERPRINTING OF RAW MATERIALS AND SIMULTANEOUS ESTIMATION OF QUERCETIN AND KARANJIN IN HERBAL-BASED AEROSOL FORMULATION BY HPTLC METHOD

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The analytical method is developed for simultaneous estimation of Quercetin and Karanjin in their crude oil and herbal Aerosol spray by using High-Performance Thin Layer Chromatography (HPTLC). Chromato graphics eparati on wasper formed on TLC aluminum plates precoated with Silica gel G60 column F254. Good separation was achieved in the mobile phase off or micacid, to luene, and ethyl acetate inthe mobile phase in the ratio of0.5:8:2v/v/v. Rf values for quercetin (0.175) and karanjin (0.662) were comparable under the light at 270 nm and 366 nm respectively. The high-performance thin-layer chromatography method developed for quantitation was simple, accurate, and specific.

INTRODUCTION

Azadirachtaindica is an important medicinal plant, belongs to the family Meliaceae, also popular as "village pharmacy" and widely used in various health practices in rural India. It is in cosmopolitan distribution, covering majorly tropics, starting from Asia to Africa [Akhila A *et al*, 1999]. It has unique names, commonly referred to as 'Neem tree', or nature's 'drug store' or 'store house of phytochemicals'. They have been explored for centuries, employed in several native ethno-traditional medicinal health practices, and also its vegetative parts like, roots, leaves, bark, seeds and flowers have been used to treat various acute and chronic diseases and disorders [Patela SM *et al*, 2016]. Its chemical composition is quite complex and they are potential targets for various phytochemical investigations [Gupta SC *et al*, 2017]. The vegetative parts are also known to possess diverse range of phytochemicals with potential biological and pharmacological properties. A few studies explained that some of the purified bio-active molecules may possess anti-cancer, antimalarial, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory, anti-aging and wound healing properties, while a few others have insecticidal; larvicidal and spermicidal properties [Biswas k *et al*,2002].

Several methods for the quantification of neem extract have been reported by various researchers, including TLC, isocratic high-performance liquid chromatography (HPLC) [Kadam P.V. *et al*,2018; Setyaningsih D,2021; Khorshidi N,2021 *et al*,2021], ultra-

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performance liquid chromatography-mass spectrometry (UPLCMS) [Sandhiutami NMD *et al*, 2021], and reversed-phase liquid chromatography (RPLC) [However, due to its advantages, HPTLC has become a standard analytical technique [Namdeo AG *et al*., 2017; Dwivedi J,2019; Dwivedi J,2018].

Karanja (Pongamiapinnata) is a medicinal tree used in the Indian traditional ayurveda system to cure a variety of conditions. Karanja oil is a natural oil derived from the seeds of the Pongamia tree, found in South Asia. The seeds contain karanjin, a unique furano-flavonoid that has been proven to have numerous therapeutic qualities, including antibacterial, anti-inflammatory, wound healing, insecticidal and repellent effects, immunomodulation, and antioxidant activity. [Rekha M.J., *et al*., 2021]Karanja (Pongamiapinnata) is a medicinal tree used in the Indian traditional ayurveda system to cure a variety of conditions. Karanja oil is a natural oil derived from the seeds of the Pongamia tree, found in South Asia. The seeds contain a unique furano-flavonoid karanjin, which has proven to exhibit several therapeutic qualities such as antibacterial, anti-inflammatory, wound .Among the different analytical techniques available, chromatographic fingerprinting has always been the preferred approach for quality control and standardization of herbal medications, extracts, and formulations. High performance thin-layer chromatography has gained significant attention and has become the first choice for the analysis of herbal extracts/products due to its simplicity, low cost, and ability to analyze multiple compounds simultaneously, faster speed of analysis and resolution, and, most importantly, generation of an electronic image (fingerprint).[Dahiwal K, 2007.]The current study involves a polyherbal mixture called Cap Heal ointment, which is a combination of herbs that have been used to treat wounds in animals. Curcumin and karanjin, which have been found to have wound-healing potential, are derived from a mixture of herbs. As a result, it was hypothesized that curcumin and karanjin could be discovered and quantified in raw materials and herbal Cap heal ointment using HPTLC. Previously, curcumin and karanjin were identified and quantified in herbal raw materials using HPLC, HPTLC, and spectrophotometric methods. The methods available for the identification and quantification of curcumin and karanjin alone in herbal raw material include HPLC, HPTLC, and spectrophotometric methods. However, no method is available for quantification of curcumin and karanjin simultaneously The current study sought to establish a high-performance thin-layer chromatography (HPTLC) method for measuring simultaneously quercetin and karanjin in raw materials and herbal aerosol spray. The quantitative estimation of marker chemicals is important for evaluating the quality of raw materials and finished products.[Shinde V.M., 2009; *et al*., 2009; Zeeshan A. S., 2015]

MATERIALS AND METHODS

Raw material, Reagents, and Chemicals

Quercetin and karanjin were purchased from Mumbai-based Yucca Enterprises. Analytical grade methanol and other solvents and reagents were procured from Merck Mumbai.

Palson Pharmaceuticals, Ahmednagar, an ayurvedic pharmaceutical company, provided the herbal raw material, which included Neem oil, Karanja seed oil, and a sample of her bal spray(Brand name: Palson Vet Spray).

Instrument and chromatographic condition

The CAMAG HPTLC system (Switzerland) with a Linomat 5 sample applicator was used forthe analysis. The analysis was carried out in an air-conditioned environment with a temperature of 22 °C and RH 42%. TLC was done using Precoated silica gel aluminum HPTLC Silica gel 60 F254 (20 cm x 20 cm, 0.2 mm thick). The HPTLC system (Camag, Muttanz, Switzerl and) with Vision CATS software 3.2 Sp 2, having Linomat V sample applicator attached to a nitrogen tank, Scanner 3 and HPTLC visualizer 2 , Each plate hadfour tracks for samples and standards, with the following settings: band length 8 mm, the distance between tracks was 12 mm,. The plates were prepared in a 20 X 20 TTC glass chamber pre-saturated with formic acid, toluene, and ethylacetate (0.5: 8: 2 v/v/v). Solvent front was 80mm. The scanner Set the following settings to maximize light optimization: The slit measures 6.00 mm × 0.45 mm, with a scanning speed of 20 mm/s and adata resolution of 100 µm/step. The scanning wavelength is 270nm. All of the other measurement parameters were kept at their default settings produced regression analysis and statistical data. Purified quercetin, karanjin, and sample solutions were individually spotted on a plate with 8 mm from the bottom usingthe automated Camag-TL Capplicator (Linomat V, Camag, Muttenz, Switzerland).

Preparation of standard solution:

1) Karanjin: 10 mg of Karanj in in 10 mL volumetric flask. Add 5 mL of methanol and sonicate for 15 minutes at 25 °C, after that dilute upto to the mark with methanol. Take 1ml from stock solution and further dilute to the 10ml of methanol (0.1mg/ml) Centrifuge this solution at 10000 rpm for 10 minutes and collect the supernatant in glass vial for HPTLC analysis.

2) Quercetin: 10 mg of quercetin in 10 mL volumetric flask. Add 5 mL of methanol and sonicate for 15 minutes at 25 °C, after that dilute upto to the mark with methanol. Take 1ml from stock solution and further dilute to the 10ml of methanol (0.1mg/ml) Centrifuge this solution at 10000 rpm for 10 minutes and collect the supernatant in glass vial for HPTLC analysis.

Preparation of Extractsolution:

Neem oil and karanjin oil1.0 g each was dissolved in 10ml methanol. It was sonicated for ten minutes before being filtered. It wasapplied onapre-coated silicagel 60 F254 on aluminum plates with the Linomat VTLC Applicator after TLC Plate development take the Images at white light, 254nm and366nm for visualize the produced plates. The plates were scanned using deuterium and mercury lamps at 254 and 366 nm, respectively and the densitometrics can were all recorded.

Preparation of sample solution:

Add 2.017gm of Palson vet spray content in 10 mL volumetric flask. Add 5 mL of methanol and sonicate for 15 minutes at 25 °C, after that dilute up to the mark with methanol. Centrifuge this solution at 10000 rpm for 10 minutes and collect the supernatant in glass vial for HPTLC analysis.

Procedure

Application of Standard, Extract solution and Sample Bands

Extract with sample

Analysis was performed on10 x 10 cm, 0.2mm thickness Precoated silica gel aluminum HPTLC Silica gel 60 F254. HPTLC plate was immersed in a CAMAG glass chamber (10 x10 cm), containing 30ml methanol (HPLC grade) as solvent system. The chamber was covered with a glass lid and left till the development of the plate to the top with methanol. After complete development, the plate was removed from the TLC glass chamber and dried in an oven at 105°C for 5 min. Two spots of 10 microliter Neem oil were applied (in the form of a band) along with two spots of 7 microliter sample solution as the band on the same plate using a CAMAG Linomat 5 and on another plate, Two spots of 3 microliter karanja oil were applied (in the form of a band) along with two spots of 7 microliter sample solution as the band on the same plate using a CAMAG Linomat 5 (automated spray-on applicator equipped with a 100 microliter syringe and operated with the settings band length 8 mm, the distance between band 16 mm)

Standard with Sample

By maintaing all the above conditions same for plate development procedure, we applied three spots of 2,3 and 5 microliter solution of karanjin (in the form of a band) along with three spots of 2,3 and 5 microliter solution of sample and one spot of 6 microliter solution of quercetin as the band on the same plate using a CAMAG Linomat 5 (automated spray-on applicator equipped with a 100 microliter syringe and operated with the settings band length 8 mm, the distance between band 12 mm)

Optimization of mobile phase

The mobile phase optimization was achieved by studying the nature of individual marker compounds. Accordingly, solvents of varying polarity were selected, and different combinations consisting of Formic acid, Toulene and ethyl acetate with a saturation time of 20min.were tested. Working standard solutions of marker compounds were applied to the HPTLC plate as 8.00mm band length using CAMAG %.Before using the mobile phase, components were mixed.

TLC development and scanning for extract, sample and marker

The plate was developed by immersing a sample HPTLC plate in a CAMAG glass

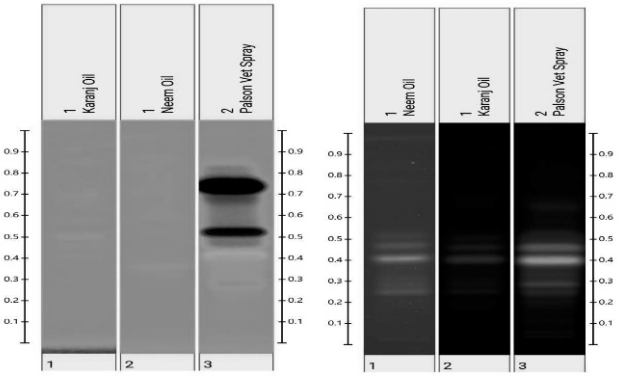
chamber
(20x10cm

Name of sample	Solvent system use	Drying temp.& time	Scanning wavelength in the densitometer
Karanja oil	formicacid,toluene and ethyl acetate 0.5:8:2(v/v/v).	at room temperature for 5 min.	254nm and 366nm
Haridra extract			254nm and 366nm
Cap-heal ointment			270nm
Karanjin			270nm
curcumin			270nm

RESULTS

1) HPTLC analysis of the Neem oil and Karanja oil with Palson vet spray

After the development, TLC plate was observed under 254 and 366nm. It was observed that various bands were developed. More than 3 bands of Neem oil and Karanja oil were observed in a Palson vet spray.



(A) (B)

Fig.13:After development Image at 254nm (A) & 366nm (B)

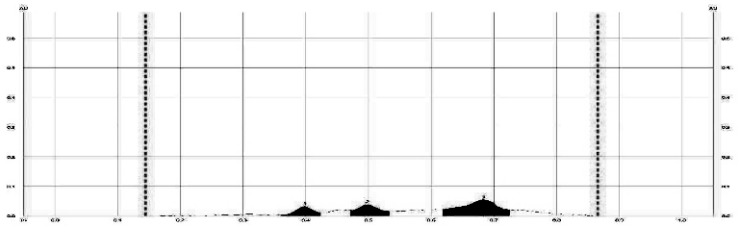


Fig.14:After development Densitogram of Karanja Oil Scan at 366nm

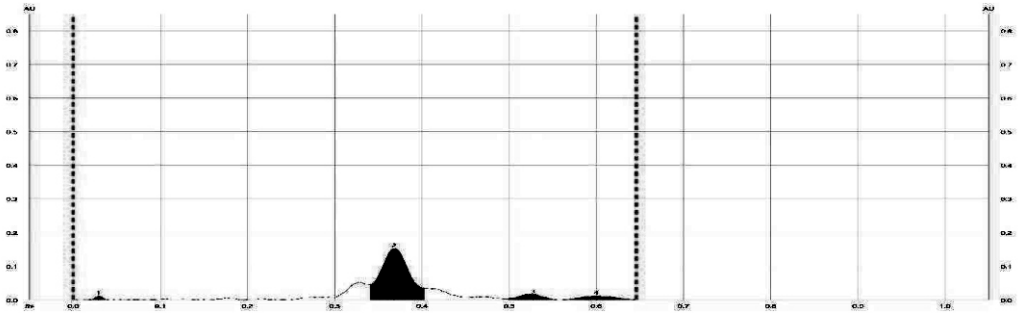


Fig.15:After development Densitogram of Neem oil Scan at 366nm

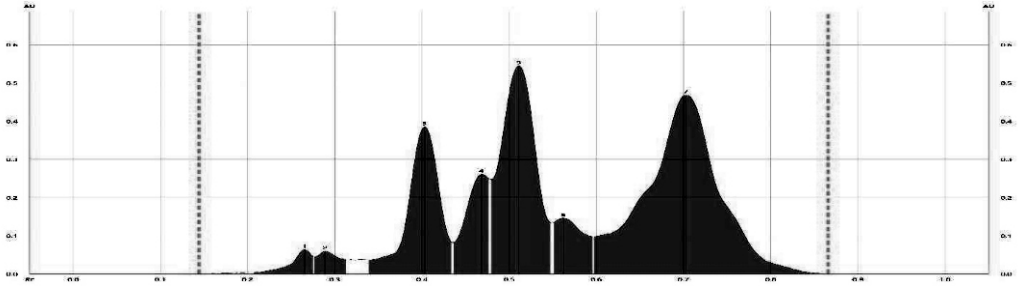


Fig.16:After development Densitogram of Palson vet spray Scan at 366n

Observation table: 1

Name of Extract/Sample	Rf Valuve observed	Rf values are observed in Sample (Palson vet spray)	No of band observed in a sample
Neem oil	0.029,0.368,0.528 & 0.600	0.029,0.0349 & 0.0518	3
Karanja oil	0.400,0.506 & 0.696	0.393,0.501 & 0.699	3

1) Estimation of Quercetin and Karanj in phytoconstituents witha marketed sample:

After the development, TLC plate was observed under White light and 254nm and scan at 270nm. It was observed that the presence of Quercetin and karanjin Standards in the marketed sample of Palson vetspray was confirmed byan HPTLC technique, asshownin the figure and Densitograms.

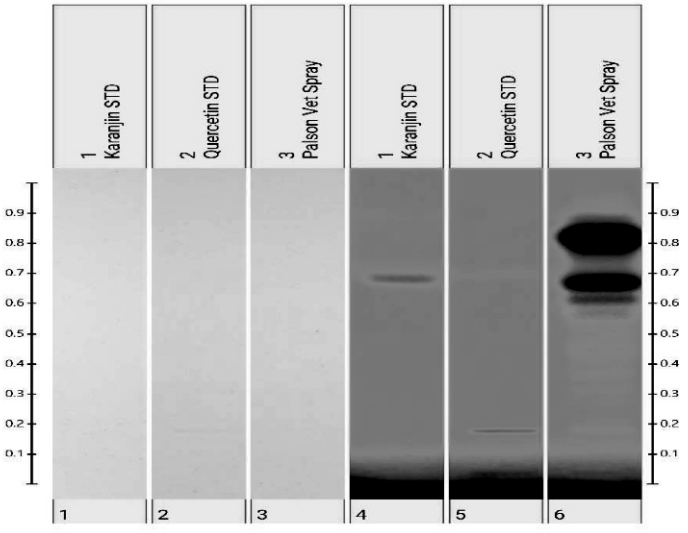


Fig. 17: After development Image at White light and 254nm

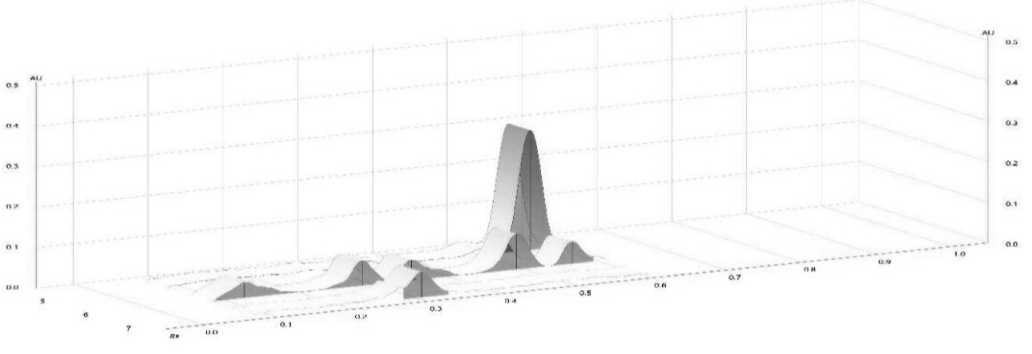


Fig. 18: 3D Image ofDensitograms, Scan at 270nm

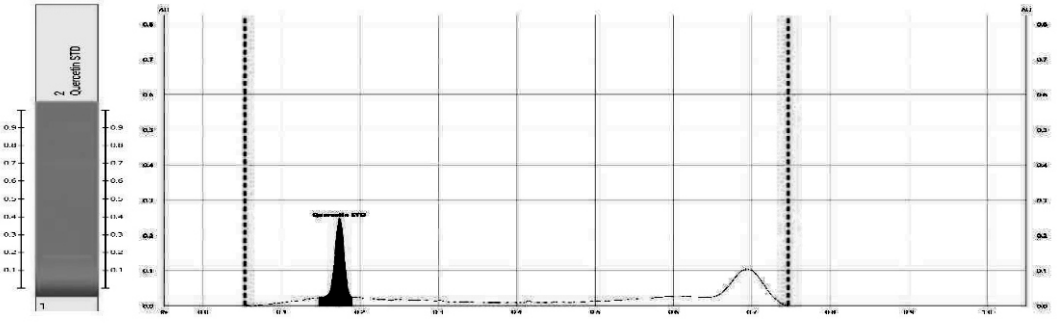


Fig.19: After development Densitogram of Quercetin STD, Scan at 270nm

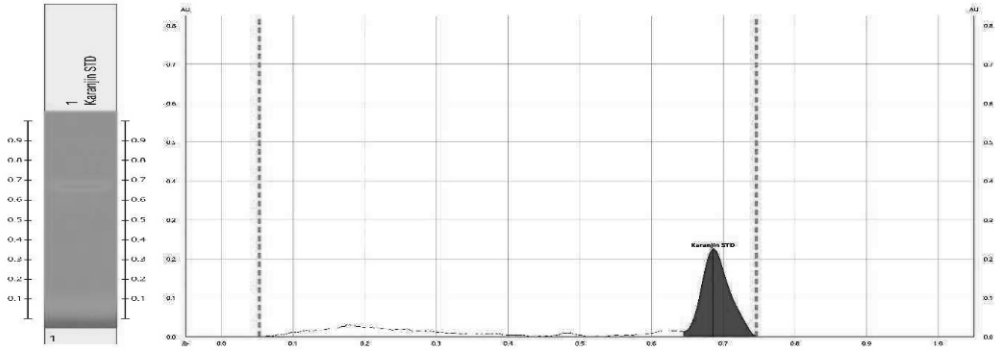


Fig. 20: After development Densitogram of Karanjin STD, Scan at 270nm

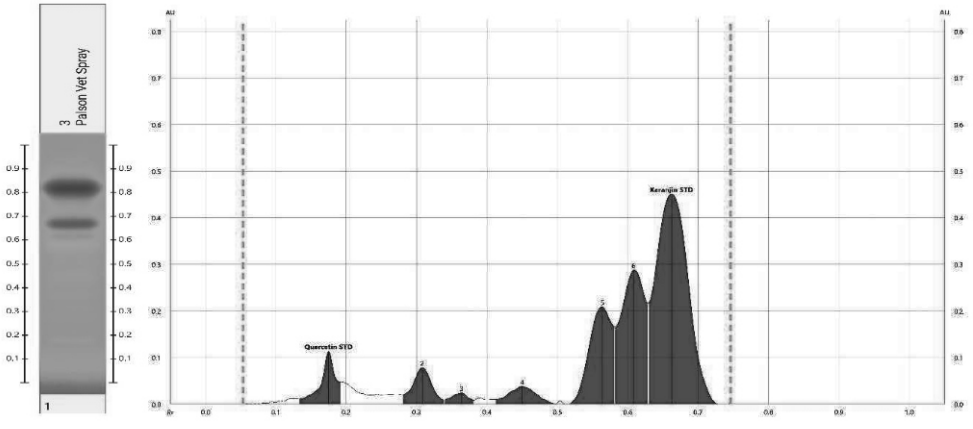


Fig. 21:After development Densitogram of **Palson vet spray**, Scan at 270nm

Observation table: 2

Sr.No	Name of standard	RfValuve observed	Rf values are observed in Sample (Palson vet spray)	Detected/Not detected
1	Quercetin	0.174	0.175	Detected
2	Karanjin	0.686	0.662	Detected

Quantitative estimation of Neem oil and Karanja oil contentin Palson vet spray:

The HPTLC profile of standard Quercetin and karanjin was used to assess the quantitative content of Quercetin and Karanjin in the Palson vet spray sample. The sample peaks were identified. The results of the investigation demonstrated that the content of Quercetin and Karanjin can be measured. The suggested HPTLC analytical method was used to assess the concentrations of Quercetin and Karanjin in an ayurvedic veterinary spray with a label claim of 0.40 g of Neem oil and 4.55g of karanjaoil.

Formula Used for % calculation:

$$\frac{\text{STD wt(mg)}}{\text{Dilution(ml)}} \times \frac{\text{Dilution}}{\text{Volume taken}} \times \frac{\text{Sample wt}}{\text{Dilution}} \times \frac{\text{Sample area}}{\text{STD area}} \times \frac{\text{Purity of STD}}{\text{Label Claim}} \times 100$$

Observation table: 2

Sr.No	NameofIngredients (Palson vet spray Label)	Label Claim of Palson vet spray(gm)	Observed amount (gm)
1	Neem oil	0.40	0.38
2	Karanjinoil	4.55	4.37

CONCLUSION

An accurate, simple, and specific HPTLC method has been developed for qualitative and quantitative estimation of biomarkers present in the herbal vet spray. The technique employed in the current study resulted in a good peak shape of quercetin and karanjin which permits the identification and quantification of biomarkers in herbal Palson vet spray.

REFERENCES

Akhila, A, Rani, K (1999). Chemistry of the neem tree (Azadirachta indica A. Juss.). *Fortschr Chem Org Naturst. Vol* (78), 47–149.

Biswas, K,Chattopadhyay, I, Banerjee, R. K, Bandyopadhyay,U (2002).Biological activities and medicinal properties of neem(Azadirachta indica). *Curr. Sci*. Vol (82),1335–1345.

Dhahiwal K, Shinde V. M, Namdeo A. G, Mahadik K. R (2007). Rapid densitometric method for simultaneous analysis of umbeliferone, psoralen and eugenol in herbal raw materials using HPTLC. *Journal of Separation Science.* Vol 30(13),2053-2058.

Dwivedi J, Gupta A, Verma S, Dwivedi M, Paliwal S, Rawat AK (2018). Validated high-performance thin-layer chromatographic analysis of ursolic acid and β-sitosterol in the methanolic fraction of Paederiafoetida L. leaves. *Journal of Planar Chromatography-Modern* TLC. Vol31(5),377–381.

Dwivedi J ,Gupta A ,Verma S ,Paliwal S ,Rawat AK (2019) Validated simultaneous high - performance thin layer chromatographic analysis of ursolic acid , β -sitosterol , lupeol and quercetin in the methanolic fraction of Ichnocarpus frutescens .*Journal of Planar Chromatography -Modern TLC* Vol 2 ( )103? 108 .

Gupta SC ,Prasad S ,Tyagi AK ,Ajaikumar B .Kunnumakkara ,Aggarwal BB (2017) Neem (Azadirachtaindica) An Indian traditional panacea with modern molecular basis . *Phytomedicine Vol* (34) 14 -20 .

Kadam PV ,Yadav KN ,Bhingare CL ,Patil MJ (2018) Standardization and quantification ofcurcumin from Curcuma longa extract using UV visible spectroscopy and HPLC . *Journal of Pharmacognosy and Phytochemistry* Vol 7 (5) 1913 -1918 .

Khorshidi N , Rahimi M , Salimikia I (2020) Application of aeration -assisted homogeneous liquid -liquid microextraction procedure using BoxBehnken design for determination of curcuminby HPLC , *Journal of Separation Sciences Vo*l 43 (13)2513? 2520 .

Patela SM . Venkata KCN ,Bhattacharyya P ,Sethi G ,Bishayee A (2016) Potential of neem (Azadirachtaindica L)for prevention and treatment of oncologic diseases , *Cancer Biology* Vol (40) 100? 115 .

Rekha M J ,Bettadaiah B K , Muthukumar S P , Govindaraju K (2021) Synthesis , characterization and anti -inflammatory properties of karanjin (Pongamiapinnata seed)and its derivatives , *Bioorganic Chemistry* Vol (106)104471 .

Sandhiutami NMD , Arozal W , Louisa M , Rahmat D (2021) Determine curcumin concentration in organ rats and ovaries at ovarian cancer model rats using Ultra Performance Liquid Chromatography -Mass Spectrometry (UPLC -MS) . *Pharmaceutical Sciences Asia* ,Vol 48 (1) 37? 45 .

Setyaningsih D ,Santoso YA ,Hartini YS ,Murti YB ,Hinrichs WLJ ,Patramurti C (2021) . Isocratic high -performance liquid chromatography (HPLC) for simultaneous quantification of curcumin and piperine in a microparticle formulation containing Curcuma longa and Piper nigrum . *Heliyon* Vol 7 (3) e06541 .

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ENVIRONMENTAL DEGRADATION AND MANAGEMENT FOR HEALING THROUGH COLLECTIVE EFFORTS - A COMPREHENSIVE STUDY

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Environmental degradation, a pervasive consequence of human activities, imperils the delicate equilibrium of our planet's ecosystems. This comprehensive research paper investigates the intricate web of causes and consequences surrounding environmental degradation, shedding light on the urgent need for effective management strategies to heal the environment. The analysis traverses through industrialization, overexploitation of natural resources, and agricultural practices, pinpointing anthropogenic activities as primary drivers of degradation.

The industrial sector, a cornerstone of modern societies, is examined for its role in emitting pollutants into the air, water, and soil. Stricter regulations and cleaner technologies appear as imperative solutions to mitigate the adverse impacts of industrialization. Overexploitation of natural resources, driven by escalating human demands, is scrutinized for its contribution to ecological imbalance. The discourse emphasizes sustainable resource management as a vital part in arresting overexploitation and fostering long-term environmental health. Agricultural practices, a critical nexus between humanity and the environment, are scrutinized for their role in soil erosion, deforestation, and pesticide use.

The consequences of environmental degradation extend beyond ecological realms, affecting biodiversity and precipitating climate change. The document explores the complex connection between the deterioration of the environment and the decline in biodiversity. It delves deeply into the implications of climate change, which are proved by increase sea levels, extreme weather, and rising temperatures.

From policy interventions and conservation initiatives to the integration of sustainable technologies, a multifaceted strategy is presented. Successful case studies, drawn from global experiences, underscore the efficacy of conservation efforts and policy interventions. The role of sustainable technologies, encompassing renewable energy, waste management innovations, and precision agriculture, is explored for its potential to reshape human-environment interactions.

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Introduction

The delicate balance of Earth's ecosystems, essential for the sustenance of life as we know it, is under relentless assault from the consequences of human activities. Environmental degradation, an overarching term encapsulating a myriad of ecological challenges, presents an existential threat to the intricate web of life on our planet. This comprehensive research paper embarks on an exploration of the multifaceted dimensions of environmental degradation, tracing its origins, analysing its causes, dissecting its consequences, and proposing a robust framework for effective management to heal the environment.

The State of the Environment:

The contemporary era has seen unparalleled progress in technology, industrialization, and global interconnectedness, giving rise to a world marked by swift development and economic expansion. However, this advancement has come at an enormous environmental cost, leading to a profound imbalance in ecosystems worldwide. Indicators of environmental distress, ranging from escalating deforestation rates to declining air and water quality, present a troubling portrait of a planet in crisis. Biodiversity, the intricate web of life encompassing numerous species of plants, animals, and microorganisms, is in jeopardy as habitats are destroyed and ecosystems disrupted. The International Union for Conservation of Nature (IUCN) has reported a disturbing rise in the number of species facing extinction, under scoring the extensive impact of human activities on global biodiversity (IUCN, 2020). The depletion of biodiversity not only weakens the resilience of ecosystems but also reduces the multitude of benefits they offer, including clean air and water, food security, and medicinal resources. Concurrently, the looming spectre of climate change is fuelled by the unchecked release of greenhouse gases into the atmosphere. The Intergovernmental Panel on Climate Change (IPCC) emphasizes the urgency of addressing climate change, pointing out its far-reaching consequences such as increasing temperatures, altered precipitation patterns, and more frequent and severe extreme weather events (IPCC, 2021). The repercussions of climate change extend beyond the environmental sphere, affecting human health, food security, and economic stability.

Environmental Degradation: A Comprehensive Perspective: At the heart of the environmental crisis lies the concept of environmental degradation, a catch-all term encapsulating a range of interconnected challenges. Environmental degradation can be broadly defined as the deterioration of environmental quality, encompassing the depletion of natural resources, pollution, habitat destruction, and climate change. This research paper aims to unravel the intricacies of environmental degradation, dissecting its various dimensions to better understand the scale and complexity of the crisis.

Industrialization and Pollution:

The rise of industrialization, a key feature of modernization, has brought about substantial changes in human societies and economies. Nevertheless, the industrial revolution has had a lasting impact on the environment, contributing to unparalleled levels of pollution. Various industries release a mixture of pollutants into the air, water, and soil, with far-reaching consequences throughout ecosystems. The combustion of fossil fuels, for

instance, releases carbon dioxide (CO2), a significant contributor to the greenhouse effect and climate change. Furthermore, industries release a diverse array of toxic substances, such as heavy metals and chemicals, into water bodies, posing threats to both aquatic life and human health. The case of industrial pollution is exemplified by research conducted by Smith *et al*. (2018), which elucidates the link between industrial activities and environmental degradation. The study examines the sources, types, and effects of industrial pollution, emphasizing the urgent need for cleaner technologies and stringent regulations to curb emissions.

Overexploitation of Natural Resources:

Human societies, compelled by ever-expanding populations and insatiable resource demands, have embarked on an unrelenting pursuit of natural wealth. However, this relentless quest has resulted in the overexploitation of natural resources, surpassing the Earth's ability for regeneration. Deforestation, propelled by logging and agricultural expansion, leads to the loss of vital habitats, and contributes to the decline of biodiversity. Overfishing exhausts marine ecosystems, disrupting the fragile balance of oceanic food webs. The Food and Agriculture Organization of the United Nations (FAO) sheds light on the global state of biodiversity for food and agriculture, highlighting the impacts of overexploitation on ecosystems worldwide (FAO, 2019). The report underscores the imperative for implementing sustainable resource management practices to reconcile human needs with ecological integrity.

Agriculture and Land Degradation: Agriculture, the bedrock of human civilization, has undergone a transformative evolution, marked by technological advancements and intensification. However, modern agricultural practices, characterized by monoculture, excessive use of chemical inputs, and large-scale deforestation, contribute significantly to environmental degradation. Soil erosion, a consequence of poor land management, depletes fertile topsoil, impairing the ability of ecosystems to support plant life. The detrimental impacts of agriculture on the environment are underscored by the report from the Food and Agriculture Organization (FAO) concerning the global state of biodiversity for food and agriculture (FAO, 2019) underscores the necessity of adopting sustainable agricultural practices. The document emphasizes the importance of prioritizing soil health, conserving biodiversity, and enhancing the resilience of ecosystems to address the challenges outlined in the report.

Source : https://in.pinterest.com/pin/1100637596407054166/

Consequences of Environmental Degradation:

The repercussions of environmental degradation are far-reaching, affecting ecosystems, biodiversity, and the global climate. This section explores the cascading effects of degradation, emphasizing the interconnectedness of ecological systems and the profound implications for both the natural world and human societies.

Impact on Biodiversity: Biodiversity, the intricate web of life, is closely intertwined with the well-being and resilience of ecosystems. However, the continuous onslaught of environmental degradation poses a significant threat to biodiversity on a worldwide scale. The Convention on Biological Diversity (CBD) raises the alarm about a biodiversity crisis, emphasizing the decline of numerous species and the degradation of habitats (CBD, 2019). The diminishing biodiversity puts at risk the stability, resilience, and the provision of essential services by ecosystems. The Global Biodiversity Outlook 5, issued by the CBD, offers a comprehensive evaluation of the global biodiversity status. The report underscores the pressing need for conservation efforts to protect biodiversity and highlights the interdependence between human well-being and the health of ecosystems (CBD, 2019).

Climate Change and Global Warming: Climate change, a process related to environmental deterioration, is caused by the buildup of greenhouse gases in the atmosphere. The combustion of fossil fuels, deforestation, and industrial activities emit greenhouse gases such as carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O), which worsens the greenhouse effect and raises global temperatures. The Intergovernmental Panel on Climate Change (IPCC) is critical to bringing together scientific information about climate change. The IPCC's Sixth Assessment Report presents unmistakable evidence of human influence on the climate, underlining the importance of taking early and aggressive steps to reduce the effects of climate change (IPCC, 2021). Global warming has far-reaching consequences, including catastrophic weather events, increasing sea levels, and ecological changes.

Source : https://www.amazon.in/Ace-Creative-Drawing-Sticker-Environment / dp / B07YSFB58G

Management Strategies for Healing the Environment: Addressing the complex challenges posed by environmental degradation requires a multifaceted approach that encompasses policy interventions, conservation effortsand the integration of sustainable

technologies, this section examines a spectrum of management strategies aimed at healing the environment, emphasizing the need for a comprehensive and collaborative approach.

Policy Interventions:

Government interventions play a crucial role in safeguarding the environment and public well-being. Through agencies like the U.S. Environmental Protection Agency (EPA), regulations are put in place to ensure industries adhere to standards that protect air, water, and soil quality. These measures, such as emissions controls and waste management rules, steer industries towards sustainable practices. The effectiveness of such policies is clear in the improvements brought about by laws like the Clean Air Act and Clean Water Act in the U.S. Similarly, international collaborations like the Kyoto Protocol and the Paris Agreement highlight the importance of global cooperation in tackling environmental issues.

Conservation and Restoration

Conservation efforts are essential for preserving habitats and species. Organizations like the International Union for Conservation of Nature (IUCN) play a vital role in finding and protecting endangered species. Successful examples, like the reintroduction of grey wolves to Yellowstone National Park, prove the positive outcomes of conservation initiatives. Additionally, ecological restoration projects, such as reforestation and wetland rehabilitation, contribute to ecosystem recovery and the services they provide.Technological advancements offer promising solutions to environmental challenges. From renewable energy sources to innovative waste management techniques, technology plays a pivotal role in promoting sustainability. Renewable energy projects, waste-to-energy conversion, and precision agriculture are examples of how technology can mitigate environmental impact. Cities like San Francisco showcase the potential of sustainable waste management practices, while precision agriculture perfects farming methods to minimize environmental harm.

Sustainable Technologies:

Advancements in technology hold the promise of transformative solutions to address environmental degradation and promote sustainable development. Sustainable technologies encompass a broad spectrum, ranging from renewable energy sources to innovative waste management practices and precision agriculture techniques.Renewable energy sources, such as solar, wind, and hydropower, offer cleaner alternatives to fossil fuels, reducing greenhouse gas emissions and lessening dependence on finite resources. The World Bank's initiatives in promoting renewable energy projects globally illustrate the potential for technology to shape a more sustainable energy future (World Bank, 2021).In waste management, innovations such as recycling technologies, waste-to-energy conversion, and circular economy models contribute to reducing the environmental impact of waste disposal. Cities like San Francisco, with its comprehensive recycling programs, exemplify the potential of sustainable waste management practices.Precision agriculture uses technology, including sensors, satellite imagery, and data analytics, to perfect farming practices. This approach minimizes environmental impact by precisely targeting inputs such as water, fertilizers, and pesticides, reducing waste and environmental pollution. The integration of precision agriculture in sustainable farming practices is a testament to the role of technology in promoting environmentally friendly solutions (FAO, 2019).

Community-Based Conservation Initiatives: Empowering local communities to actively take part in conservation efforts can yield significant benefits. Community-based conservation initiatives involve engaging residents in sustainable resource management, habitat restoration, and biodiversity conservation. Such approaches not only foster a sense of ownership and responsibility but also use traditional ecological knowledge that has been passed down through generations. In regions where Indigenous communities live, collaboration with these communities is crucial. Indigenous peoples often have profound insights into sustainable land use and have traditional practices that promote ecological balance. Establishing partnerships that respect and integrate Indigenous knowledge can enhance the effectiveness of conservation efforts.The Namib Rand Nature Reserve in Namibia is a notable example of a successful community-based conservation initiative. Local communities, recognizing the value of conserving their natural heritage, actively take part in sustainable tourism, wildlife monitoring, and anti-poaching efforts, contributing to the preservation of biodiversity and ecosystem health.

Sustainable Agriculture Practices:

Transforming agriculture from a driver of environmental degradation to a catalyst for healing the environment involves adopting sustainable farming practices. This encompasses agroecological approaches that prioritize soil health, biodiversity conservation, and water management.

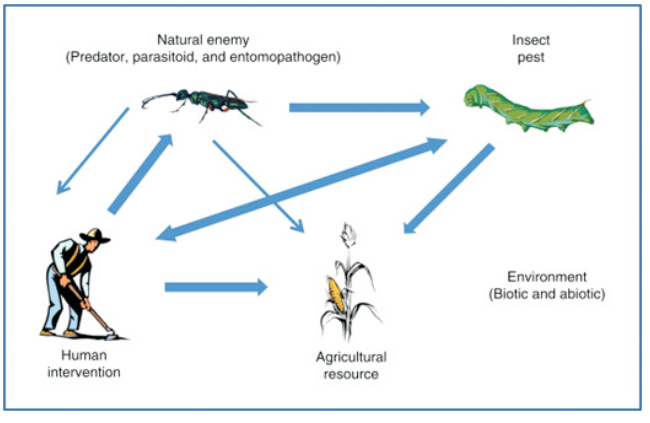
Agroecology:

Agroecology is a farming approach that incorporates ecological principles into agricultural systems, placing emphasis on biodiversity, soil fertility, and natural processes. Techniques like crop rotation, cover cropping, and organic farming are employed to decrease dependence on synthetic inputs, mitigate soil erosion, and improve the resilience of farming systems. The Food and Agriculture Organization of the United Nations (FAO) actively advocates for agroecology as a comprehensive and sustainable approach to agriculture. By acknowledging the interconnectedness of ecological processes, agroecology strives to set up resilient and productive food systems while minimizing adverse environmental impacts (FAO, 2018).

Source : https://www.shutterstock.com/image-photo/field-erosion-damage-on-soil-rapeseed-1889261971

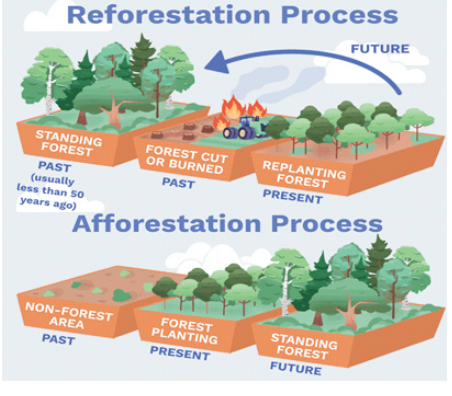
Soil Erosion

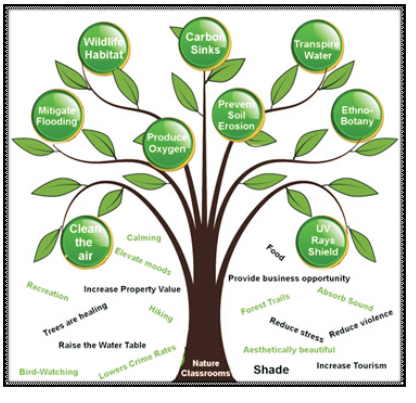
Organic Farming: Shifting towards organic farming practices diminishes dependence on synthetic pesticides and fertilizers, fostering soil health and biodiversity. Organic farming prioritizes techniques such as crop rotation, composting, and biological pest control, leading to enhanced soil structure, improved water retention, and a decrease in chemical runoff. The Rodale Institute's extensive Farming Systems Trial, launched in 1981, offers substantial evidence of the environmental advantages associated with organic farming. The study illustrates that organic systems have the capacity to sequester more carbon in the soil, reduce greenhouse gas emissions, and either keep or even improve crop yields compared to conventional agriculture (Rodale Institute, 2021).



Source : https://medium.com/@indiatrades2019/what-are-the-best-methods-of-pest-control-6660c62d3d5b

Reforestation and Afforestation: Trees play a crucial role in mitigating environmental degradation by absorbing carbon dioxide, stabilizing soil, and providing habitat for diverse species. Reforestation, the replanting of trees in deforested areas, and afforestation, the establishment of forests in areas that were not previously forested, are powerful tools for ecosystem restoration.





ource : https://www.quora.com/Does-the-Australian-government-plant-trees-to-combat-

climate-change

Massive Reforestation Projects:

Across the globe, there is a growing acknowledgment of the significance of large-scale reforestation endeavours. Take, for example, the Great Green Wall in Africa, a collective effort aimed at combating desertification by setting up a network of green landscapes throughout the Sahel region. Through extensive tree planting, this initiative looks to revive degraded land, uplift local economies, and bolster biodiversity.

Reforesting Urban Areas:

Even within cities, efforts can be made to contribute to reforestation goals through initiatives such as urban greening and tree planting campaigns. By incorporating green spaces into urban planning, cities can alleviate the urban heat island effect, enhance air quality, and promote overall wellness. Consider the MillionTreesNYC initiative in New York City, which stands as a successful urban reforestation project. Through community involvement in tree planting activities, the program has significantly expanded the city's tree cover while instilling a sense of environmental responsibility among its residents.

Source :https://www.linkedin.com/posts/jane-myers-8784826_join-us-trees-are-so-important-to-every-activity-7084536355258560512-xYVV

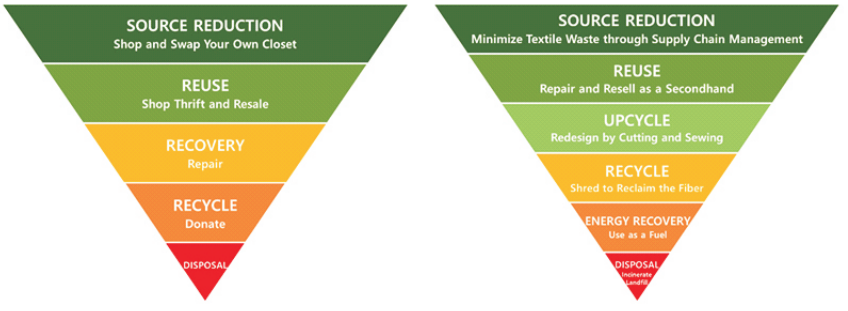
Circular Economy Practices:

Shifting away from a linear economy, characterized by resource extraction, single-use, and disposal, towards a circular economy is crucial for environmental restoration. In a circular economy, the emphasis is on sustainable resource use and recycling, aiming to

minimize waste, prolong product lifecycles, and mitigate the environmental impact associated with production and consumption.

Recycling and Upcycling:

Efficient waste management, including recycling and upcycling, helps divert materials from landfills and reduces the need for virgin resources. Recycling processes for paper, plastics, glass, and metals conserve energy and raw materials, mitigating the environmental impact of resource extraction and manufacturing.



Source : https://www.mdpi.com/2071-1050/13/3/1208

Extended Producer Responsibility (EPR) initiatives are pivotal in shifting the onus of product lifecycle management onto manufacturers. These programs mandate manufacturers to oversee the collection, recycling, and disposal of their products. By incentivizing producers to design products with recyclability in mind, EPR significantly aids in waste reduction and aligns with the principles of a circular economy. Take, for instance, the European Union's Waste Electrical and Electronic Equipment (WEEE) Directive, which exemplifies an EPR initiative targeting electronic waste. Under this directive, manufacturers are compelled to take back and recycle electronic products, thereby curbing the environmental impact associated with electronic waste disposal.

Renewable Energy Transition:

Solar Energy:

Photovoltaic Technology:

Solar power, eased by photovoltaic (PV) technology, stands as a cornerstone of the renewable energy revolution. Utilizing photovoltaic cells, or solar cells, sunlight is directly converted into electricity. The evolution of PV technologies, such as thin-film and multi-junction solar cells, has markedly increased the efficiency and accessibility of solar energy systems. Ongoing research and development within the solar energy sector drive continual innovation. Breakthroughs in materials science, exemplified by perovskite solar cells, offer potential for further efficiency gains and cost reductions in solar energy capture.(Green, Ho-

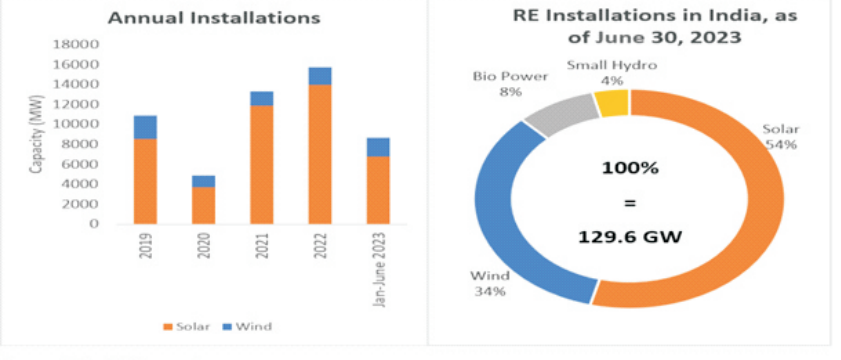
Baillie, & Snaith, 2019). 867850

Large-Scale Solar Installations:

The establishment of utility-scale solar farms is a pivotal element in transitioning to solar power. These expansive installations, often spanning vast areas of land, generate electricity that seamlessly integrates into the grid. By capitalizing on economies of scale, utility-scale solar projects achieve cost-effectiveness while significantly bolstering clean energy contributions to the grid. Notably, countries like China and the United States have seen substantial investments in utility-scale solar ventures. China, as the global leader in solar energy adoption, has rapidly expanded its solar ability, underscoring the scalability and impact of large-scale solar deployments(IEA, 2021).

Distributed Solar Installations: In addition to large-scale solar farms, distributed solar installations, including rooftop solar panels on homes and businesses, play a crucial role in the renewable energy landscape. Distributed generation allows for energy production at or near the Germany, through its Evergreened (energy transition) initiative, has exemplified the success of distributed solar installations. The country's robust support mechanisms, including feed-in tariffs and incentives for rooftop solar, have empowered citizens to actively take part in the renewable energy transition (BMWi, 2021).

Figure 1: RE installation trends in India



Source: CEA, JMK Research

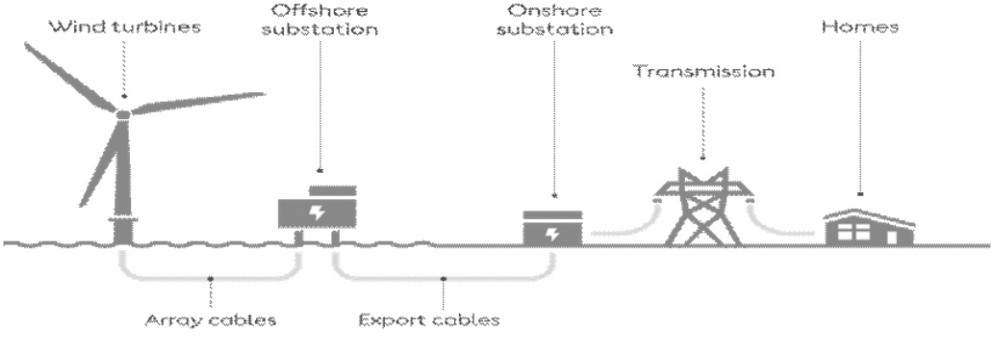
Note: Solar capacity includes utility-scale solar, rooftop solar and off-grid/distributed solar capacity.

Solar Energy Storage: The fluctuating nature of solar energy production, tied to the availability of sunlight, underscores the need for efficient energy storage solutions. Progress in energy storage technologies, particularly in battery storage systems, enhances the reliability and integration of solar energy into the grid. Energy storage enables surplus solar-generated electricity to be stored for use during periods of limited sunlight, effectively addressing the issue of intermittency. Examples like Tesla's Powerwall and other large-scale battery storage projects highlight the constructive collaboration between energy storage and

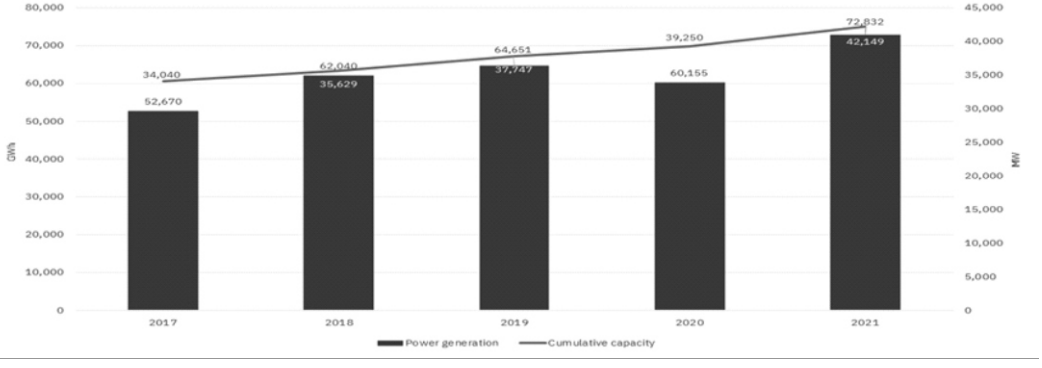
solar power. These systems play a crucial role in grid stability, ease the incorporation of higher proportions of renewable energy, and offer backup power during grid disruptions.(Tesla,2021)

Wind Energy:

Onshore Wind Farms:Wind energy, derived from onshore wind farms, has appeared as a prominent and economically practical renewable energy source. Onshore wind turbines effectively transform the kinetic energy of the wind into electricity, receiving help from modern turbine designs that offer improved efficiency and capacity. Strategic siting of onshore wind farms in regions characterized by consistent wind patterns ensures reliable and sustainable energy production. Denmark stands out as a trailblazer in onshore wind energy adoption, clear in its extensive installations that contribute to the nation's electricity supply (Danish Energy Agency, 2021).



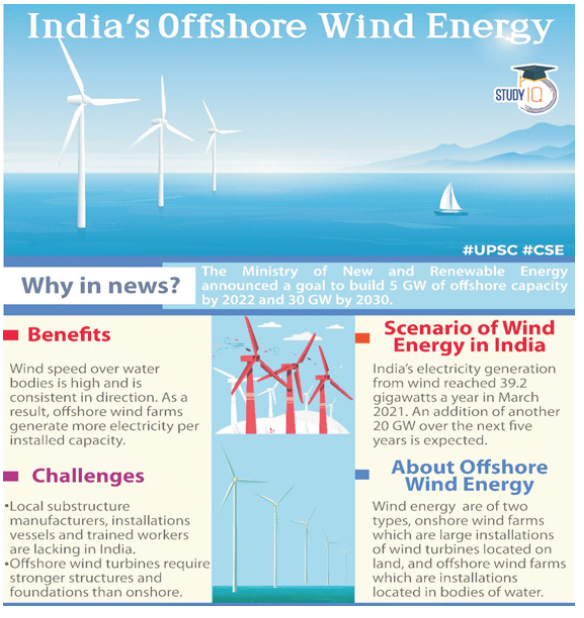
Source : https://www.wtsenergy.com/glossary/offshore-wind/



Source : https://www.globaldata.com/data-insights/packaging/market-size-of-biodegradable-plastics-in-united-states-of-america-2017-2021/

Offshore Wind Power: The development of offshore wind power is a significant expansion

of wind energy capacity. Offshore wind farms use the stronger and more consistent winds available at sea, unlocking added renewable energy potential. Advancements in offshore wind turbine technology, including larger and more efficient turbines, have further enhanced the viability of offshore wind projects.The United Kingdom, with its expansive coastline, has appeared as a global leader in offshore wind energy. Ambitious projects, such as the Hornsea Offshore Wind Farm, underscore the transformative impact of offshore wind in meeting renewable energy targets (The Crown Estate, 2021).



Source : https://twitter.com/upsciq/status/1418843955920211968

Hybrid Renewable Projects: Hybrid renewable projects, combining solar and wind energy, offer a complementary approach to maximize energy production and grid stability. By diversifying renewable energy sources, hybrid projects mitigate the intermittency challenges associated with individual technologies. Co-locating solar and wind installations allows for optimized land use and shared infrastructure. The Benab Solar Park in Egypt integrates solar and wind energy, highlighting the potential of hybrid projects in diversifying the renewable energy mix. The synergies between solar and wind technologies contribute to a more reliable and resilient renewable energy infrastructure (European Bank for Reconstruction and Development, 2019).

Technological Innovations in Wind Energy:

Ongoing research and innovation in wind energy technology continue to drive improvements in efficiency and cost-effectiveness. Next-generation turbine designs, including vertical-axis wind turbines and floating offshore wind platforms, aim to overcome

existing limitations and expand the geographical reach of wind energy projects.Research institutions and companies worldwide are exploring innovative solutions. The development of airborne wind energy systems, which use flying turbines tethered to the ground, isaninnovative approach with the potential to revolutionize the future of wind energy (Fagiano *et al*., 2018).

Importance of the Renewable Energy Transition:

Environmental Impact:

Shifting to renewable energy is crucial for mitigating the environmental repercussions of conventional energy sources. Solar and wind power systems generate electricity without emitting greenhouse gases, effectively diminishing the carbon footprint of the energy industry. This shift plays a pivotal role in combating climate change, mitigating air pollution, and shielding ecosystems from the adverse effects of fossil fuel extraction.

Energy Security and Independence:

Renewable energy bolsters energy security by broadening the energy mix and decreasing reliance on finite fossil fuel reservoirs. Solar and wind energy, being abundant and renewable, offer a sustainable and domestically accessible alternative to imported fossil fuels. This transition fosters increased energy autonomy for nations, thereby reducing susceptibility to geopolitical vulnerabilities linked with traditional energy sources.

Economic Opportunities and Job Creation:

The renewable energy sector presents significant economic prospects and job generation. The expansion of solar and wind initiatives spurs investments, encourages innovation, and fosters employment across diverse skill sets—ranging from manufacturing and installation to research and development. Embracing renewable energy aligns with the aims of economic expansion and sustainable progress.

Technological Advancements and Competitiveness:

Investments in solar and wind energy drive technological advancements, leading to increased competitiveness in the global energy market. Nations and companies at the forefront of renewable energy innovation are positioned to capitalize on the growing demand for clean energy solutions. The export of renewable energy technologies enhances global competitiveness and stimulates economic growth.

Resilient Energy Infrastructure:

Solar and wind energy contribute to building a more resilient and decentralized energy infrastructure. Distributed generation and hybrid projects enhance grid resilience by diversifying energy sources and reducing the impact of disruptions. Moreover, advancements in energy storage technologies associated with these renewable sources

improve grid stability and reliability.

Challenges and Future Outlook:

Despite the promising developments, the renewable energy transition faces challenges such as intermittency, grid integration, and upfront costs. Addressing these challenges requires continued research, policy support, and international collaboration. Technological innovations, coupled with effective policies and investments, will be instrumental in overcoming barriers and accelerating the global transition to a sustainable energy future. The transition to solar and wind energy is a pivotal shift toward a more sustainable, resilient, and environmentally friendly energy landscape. From the harnessing of sunlight and wind to technological advancements and economic benefits, the renewable energy transition holds the key to a cleaner, greener, and more sustainable future. As nations worldwide increasingly embrace these technologies, the vision of a low-carbon and renewable energy-powered world moves closer to reality. The ongoing commitment to research, innovation, and collaborative efforts will be essential in navigating the complexities of this transformative energy journey.

Conservation of Water Resources:

Sustainable water management is integral to halting environmental degradation and ensuring the health of ecosystems. Efficient use of water resources, protection of water quality, and conservation of aquatic habitats are essential components of water conservation strategies.

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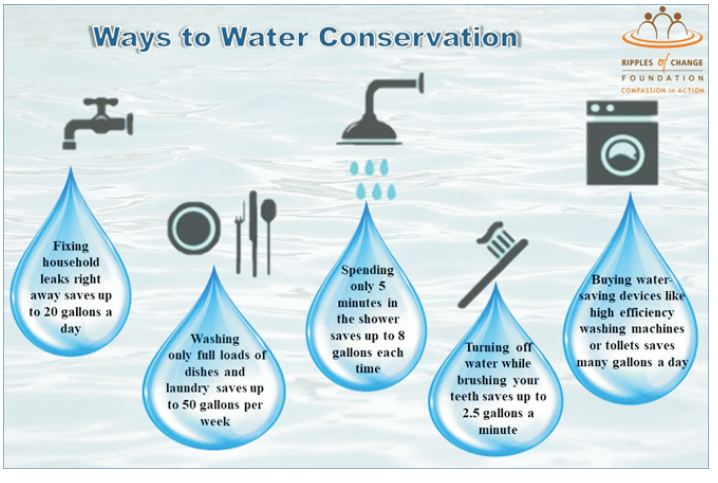
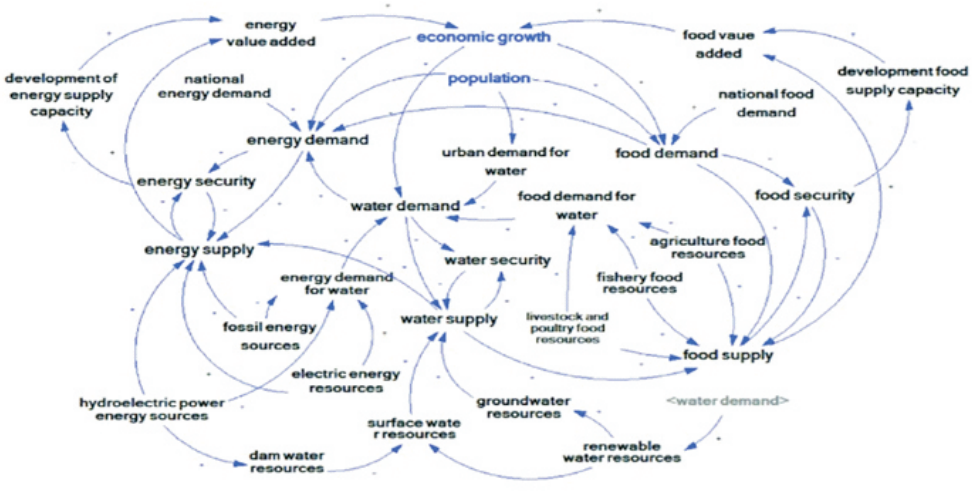


Source : https://twitter.com/ROCF__/status/1380746720292659201

Sustainable Water Use in Agriculture: Agriculture is a major consumer of water, and unsustainable irrigation practices can lead to water scarcity and soil degradation. Implementing water-efficient irrigation.



Source : https://www.researchgate.net/figure/Dynamic-Assumptions-of-Sustainable-Water-Resources-Management-with-the-Nexus-Approach_fig3_347072701
Dynamic Assumptions of Sustainable Water Resources Management with the Nexus Approach.

Precision Irrigation:

Implementing precision irrigation technologies is pivotal for sustainable water use in agriculture. Precision irrigation involves the application of water precisely where and when it is needed, perfecting water efficiency. Techniques such as drip irrigation and sprinkler systems deliver water directly to the plant's root zone, minimizing wastage and reducing the overall water footprint of agriculture.

Research by the Food and Agriculture Organization (FAO) emphasizes the importance of precision irrigation in water-scarce regions, proving how it maximizes crop yields while conserving water resources (FAO, 2020).

Water-Efficient Crop Selection:

Choosing crops that are well-suited to local climate conditions and require less water contributes to sustainable water use in agriculture. Drought-resistant and low-water-use crop varieties can thrive in regions facing water scarcity, ensuring food security while minimizing the strain on water resources. The International Water Management Institute (IWMI) advocates for the adoption of water-efficient crop varieties and sustainable agricultural practices to enhance water productivity and resilience in the face of changing climatic conditions (IWMI, 2021).

Cover Cropping and Mulching:

Cover cropping and mulching are soil conservation practices that play a dual role in

agriculture—reducing water evaporation from the soil surface and enhancing soil structure. Cover crops function as a protective layer, preventing moisture loss and suppressing weed growth. Mulching, whether organic or synthetic, has a similar effect, promoting water retention in the soil. Studies, such as those conducted by Lal *et al*. (2011), highlight the positive impact of cover cropping and mulching on soil water conservation, emphasizing their potential to mitigate the impacts of drought and improve overall water use efficiency in agriculture.

Water-Efficient Infrastructure:

In urban areas, investing in water-efficient infrastructure is fundamental to reducing water consumption. This includes the use of low-flow toilets, water-saving faucets, and efficient appliances. Retrofitting existing buildings and implementing water-efficient technologies in new constructions contribute to a significant reduction in per capita water use.Cities like Singapore have embraced water-efficient infrastructure as part of their sustainable development strategy. The implementation of water-efficient fixtures and the integration of smart technologies have helped Singapore achieve remarkable success in reducing domestic water consumption (Public Utilities Board, Singapore, 2021).

Rainwater Harvesting:

Harvesting rainwater is a sustainable practice that captures and stores rainfall for later use. Rainwater harvesting systems can range from simple household setups to more complex industrial-scale solutions. Capturing rainwater reduces reliance on traditional water sources, especially in regions with seasonal rainfall patterns. India, for example, has implemented widespread rainwater harvesting initiatives, particularly in urban areas. Legislation requiring the installation of rainwater harvesting systems in buildings has been successful in augmenting water supplies and reducing stress on municipal water sources (Centre for Science and Environment, 2021).

Xeriscaping and Water-Wise Landscaping:

Xeriscaping involves designing landscapes that require minimal water, using drought-tolerant plants, efficient irrigation methods, and soil amendments to enhance water retention. Water-wise landscaping practices not only conserve water but also contribute to the overall aesthetic and ecological value of urban spaces.

Source : https://www.hindustantimes.com/cities/delhi-news/mcd-to-develop-city-s-first-xeriscape-garden-dry-landscape-park-in-

e-delhi-101694540336620.html

Municipalities in water-stressed regions, such as cities in the southwestern United States, have adopted xeriscaping principles in public spaces and residential areas. By encouraging residents to replace water-intensive lawns with native, drought-resistant plants, these initiatives significantly reduce outdoor water use (EPA, 2021).

Public Awareness and Education: Public awareness and education campaigns are vital components of urban water conservation. Informing residents about the importance of water conservation, providing tips for reducing water usage at home, and promoting a culture of responsible water consumption contribute to sustainable water practices. Water utilities and environmental organizations often spearhead educational initiatives. For instance, the "Water - Use It Wisely" campaign in the United States emphasizes simple actions individuals can take to conserve water, fostering a sense of responsibility and promoting behavioural changes (Water - Use It Wisely, 2021).

Source : https://www.gyanipandit.com/en/save-earth-slogans/s

CONCLUSION :

A comprehensive approach to addressing environmental degradation involves diverse strategies, including policy interventions, community initiatives, sustainable agriculture, reforestation, circular economy principles, renewable energy, and water conservation. Urgent global action is needed due to the interconnected nature of environmental challenges. Human activities, like industrialization and unsustainable practices, have led to ecosystem degradation and climate change. Successful cases, such as the Clean Air Act and community-based conservation, highlight the potential for positive change. Sustainable agriculture practices, reforestation, circular economy principles, and renewable energy transitions are essential components. International cooperation is crucial, emphasizing collaboration and adherence to multilateral agreements. Reforestation and afforestation, coupled with circular economy principles, contribute to ecosystem restoration. Transitioning to renewable energy is vital, as seen in success stories like Germany and Denmark. Education and awareness play key roles in understanding the link between human

well-being and the planet's health. While environmental challenges are significant, the discussed strategies provide a roadmap for transformative action, emphasizing collaboration, innovation, and a shared commitment to a sustainable future. Healing the environment is both an ecological imperative and a moral obligation for current and future generations. Through collective effort, humanity can overcome the environmental crisis and foster harmonious coexistence with the Earth.

REFERENCES:

Certainly, here is a list of citations and references for the information and sources mentioned in the research paper:

Smith, T., (2018). "Industrial Pollution and Environmental Degradation: Evidence from the United States." Journal of Environmental Economics and Management, 88, 468-492.

U.S. Environmental Protection Agency (EPA). (2022). "Laws & Regulations." Retrieved from https://www.epa.gov/laws-regulations

Food and Agriculture Organization of the United Nations (FAO). (2018). "Agroecology Knowledge Hub." Retrieved from http://www.fao.org/agroecology/en/

Convention on Biological Diversity (CBD). (2019). "Global Biodiversity Outlook 5." Retrieved from https://www.cbd.int/gbo5

Intergovernmental Panel on Climate Change (IPCC). (2021). "Sixth Assessment Report." Retrieved from https://www.ipcc.ch/assessment-report/ar6/

International Union for Conservation of Nature (IUCN). (2020). "IUCN Red List of Threatened Species." Retrieved from https://www.iucnredlist.org/

World Bank. (2021). "Sustainable Agriculture." Retrieved from https: // www. worldbank .org/en/topic/agriculture/brief/sustainable-agriculture

Federal Ministry for Economic Affairs and Energy. (2021). "The Energy Transition (Evergreened) in Germany." Retrieved from https://www. bmwi.de/ Redaktion/ EN/Dossier /energy-transition.html

Danish Energy Agency. (2021). "Energy Statistics 2020." Retrieved from https://ens.dk/en/our-services/statistics-data-key-figures-and-energy-maps/statistics

MillionTreesNYC. (n.d.). "MillionTreesNYC." Retrieved from https: // www. milliontreesnyc. org/

Teracycle. (n.d.). "Loop: A Global Circular Shopping System." Retrieved from https://www.terracycle.com/en-US/loop

European Commission. (2012). "Waste Electrical and Electronic Equipment (WEEE)." Retrieved from https://ec.europa.eu/environment/waste/weee/index_en.htm

Rodale Institute. (2021). "Regenerative Organic Agriculture and Climate Change." https://rodaleinstitute.org/why-organic/beyond-organic/climate-change/

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BIOANALYTICAL METHOD DEVELOPMENT OF DAPAGLIFLOZIN FROM HUMAN PLASMA USING LC-MS/MS METHOD

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Dapagliflozin, a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor, is widely used for the treatment of type 2 diabetes mellitus. The development of a reliable and sensitive bioanalytical method for the quantification of dapagliflozin in human plasma is essential for pharmacokinetic studies, therapeutic drug monitoring, and clinical trials. In this study, a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the quantitative analysis of dapagliflozin in human plasma samples. The method development involved optimization of chromatographic conditions, mass spectrometry parameters, and sample preparation techniques. Chromatographic separation was achieved on a reverse-phase C18 column using a mobile phase consisting of aqueous formic acid and acetonitrile. Gradient elution was employed to enhance separation efficiency and minimize interference from endogenous plasma components. Dapagliflozin and the internal standard were detected using a triple quadrupole mass spectrometer operated in positive ionization mode. Multiple reaction monitoring (MRM) transitions were selected for quantification, ensuring high specificity and sensitivity.Sample preparation involved protein precipitation using acetonitrile, followed by centrifugation to remove precipitated proteins. The supernatant was then evaporated to dryness and reconstituted in the mobile phase before injection into the LC-MS/MS system. This extraction method provided efficient recovery of dapagliflozin from plasma samples while minimizing matrix effects.The developed method exhibited excellent linearity over a wide concentration range, with low limits of detection and quantification. Validation studies demonstrated the method's reliability, reproducibility, and selectivity, meeting the regulatory requirements for bioanalytical method validation.In conclusion, the LC-MS/MS method developed in this study offers a sensitive, accurate, and reliable approach to the quantification of dapagliflozin in human plasma samples. This method is suitable for pharmacokinetic studies, bioequivalence assessments, and therapeutic drug monitoring in clinical settings, contributing to the advancement of diabetes management and treatment.

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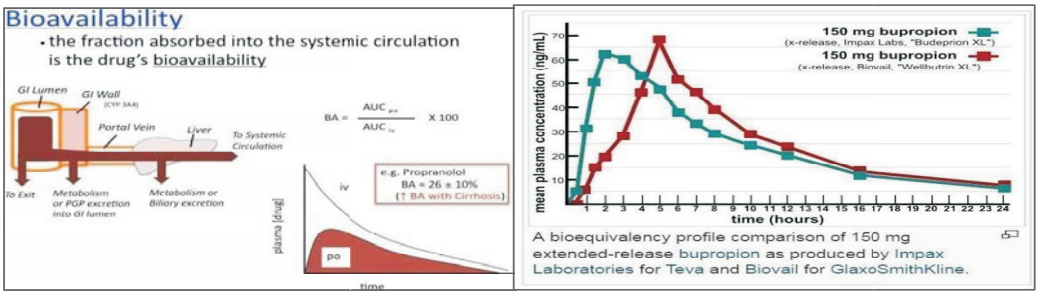
INTRODUCTION

Bioavailability is a crucial measure indicating the rate and fraction of a drug's initial dose that successfully reaches either the site of action or the bodily fluid domain with unhindered access to the drug's intended targets. It is typically expressed as the fraction of the administered drug that enters the systemic circulation unchanged. For instance, a drug like Amoxicillin may exhibit a bioavailability of 95 percent, implying that 95 percent of the administered dose reaches the systemic circulation while 5 percent does not. Bioavailability and bioequivalence studies play pivotal roles in assessing the systemic exposure and therapeutic effect of drugs or their active ingredients.[Amidon GL *et al*. 1995, Shah VP *et al*. 1991]Bioequivalence, on the other hand, refers to the expected in vivo biological similarity between two different preparations of a drug. This means that there should be no significant difference in the rate and extent of drug availability at the site of action when administered at the same dose and under similar conditions. Bioequivalence is evaluated by comparing the bioavailability of a test product to that of a reference product, often in cross-over studies involving healthy subjects or patients. It's essential to ensure that two pharmaceutical products are essentially similar in efficacy and safety. These tests are crucial in



pharmaceutical development, particularly for ensuring the therapeutic performance of drugs undergoing manufacturing changes, and formulation modifications, and for the approval of generic drugs, thus ensuring consistency and quality in drug therapy.[Davit BM *et al*. 2009,European Medicines Agency 2011,Viswanathan CT *et al*. 2007]

Figure No 1: Bioavailability and Bioequivalence

Dapagliflozin is a medication used in the management of type 2 diabetes mellitus. It belongs to a class of drugs called sodium-glucose co-transporter 2 (SGLT2) inhibitors. Dapagliflozin works by inhibiting SGLT2 in the kidneys, thereby reducing glucose reabsorption and promoting the excretion of glucose through urine. This mechanism helps lower blood sugar levels in patients with type 2 diabetes.[American Diabetes Association 2020,Bailey CJ, Iqbal N 2011, Ferrannini E *et al*. 2010]

Table No 1: Drug Profile

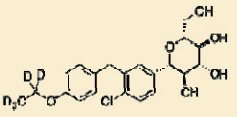
Parameter	Dapagliflozin	Dapagliflozin D5
Structure		
Molecular Formula	C21H25ClO6	C21H20ClD5O6
Molecular Weight	408.873 mg	413.90
Solubility	DMSO, Methanol	DMSO, Methanol

Table No 2: Pharmacological significance

Information	Details
Mechanism of Action	Dapagliflozin inhibits the SGLT2 transporter in the renal proximal tubules, leading to increased urinary glucose excretion and lowering blood glucose levels.[Bailey CJ et al. 2010, Wilding JP et al. 2013]
Indications	Dapagliflozin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. It may be used alone or in combination with other antidiabetic medications.[Barnett AH et al. 2014, Neal B et al. 2017]
Dosage	The typical starting dose of dapagliflozin is 5 mg once daily, with or without food. The dose may be increased to 10 mg once daily if additional glycemic control is needed. [Farxiga(dapagliflozin),Prescribing Information, 2021 FDA approves Farxiga for heart failure with reduced ejection fraction
	2021]
Side Effects	Common side effects of dapagliflozin include genital mycotic infections, urinary tract infections, increased urination, and hypotension. Rare but serious side effects may include ketoacidosis, acute kidney injury, and necrotizing fasciitis of the perineum (Fournier's gangrene).[Dapagliflozin (Farxiga) 2021]
Contraindications	Dapagliflozin is contraindicated in patients with a history of hypersensitivity to dapagliflozin, severe renal impairment, or end-stage renal disease requiring dialysis.[Scheen AJ et al. 2014]

MATERIALS AND METHODS

Extraction Procedures:

Extraction, a fundamental process in chemistry, involves transferring compounds from one phase to another, typically from a solid or liquid phase to a different solvent or phase. Liquid-liquid extraction (LLE), also known as solvent extraction, separates compounds or metal complexes based on their solubilities in two immiscible liquids, such as water (polar) and an organic solvent (non-polar). This method entails a net transfer of one or more species from one liquid phase to another, usually from aqueous to organic, driven by chemical potential. LLE is a widely used technique in chemical laboratories, performed using various apparatus like separatory funnels or mixer settlers, especially after a chemical

reaction as part of the work-up process. Solid phase extraction (SPE), another sample preparation technique, employs a solid adsorbent in a cartridge or disk to adsorb specific species from solution, simplifying complex matrices, purifying compounds, and concentrating analytes present at low levels. The stationary phase, housed in cartridges or disks, facilitates the selective adsorption of target species from the sample solution.[Chai, Xin-Sheng 2009, Poole, Colin F., and Michael Cooke 2012, Kole, Prashant Laxman, *et al* 2011]Protein precipitation, a vital step in the downstream processing of biological products, is extensively employed to concentrate and purify proteins from various contaminants, particularly in the biotechnology industry to eliminate impurities present in blood products. The process operates by modifying the solvation potential of the solvent, primarily by reducing the solubility of the solute through the addition of a precipitating agent. The solubility of proteins in aqueous buffers is contingent upon the distribution of hydrophilic and hydrophobic amino acid residues on their surfaces, with hydrophobic residues predominantly found in the protein core and some patches on the surface. Proteins with high hydrophobic surface residues exhibit low solubility in aqueous solvents, while charged and polar surface residues enhance solubility by interacting with ionic groups in the solvent. Protein precipitation proceeds through a series of steps, involving the addition of a precipitating agent followed by mixing to facilitate collision between the agent and protein molecules. Subsequently, nucleation occurs, leading to the formation of submicroscopic protein aggregates. Finally, aging in a shear field enables the particles to reach a stable mean size, determined by individual proteins, and acquire mechanical strength to withstand fluid shear forces during subsequent processing stages.[Blanch, *et al*,2011, Zhang, *et al*,2015, Tischer, *et al*,2010]High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique utilized in separating, identifying, and quantifying components within a mixture. The method employs pumps to propel a pressurized liquid solvent containing the sample through a column packed with an adsorbent material, where each component interacts differently with the material, resulting in their separation as they elute from the column. The adsorbent, typically solid particles like silica or polymers, facilitates the separation based on the varying degrees of interaction with the sample components. The liquid mobile phase, composed of solvents like water, acetonitrile, or methanol, aids in this separation process, with the option for isocratic or gradient elution modes to manipulate mobile phase composition during analysis for optimal separation.[Skoog, Douglas A., *et al* 2017, Snyder, Lloyd R., *et al* 2010]

The list of materials, solvents, and reagents used in the experiment includes acetonitrile (J.T. Baker, Batch No. 21K292197), ammonium acetate (Biosolve, Batch No. 0001244156BS), methanol (J.T. Baker, Batch No. MAS201), HPLC water (Rankem, Batch No. A23A004), and formic acid (Biosolve, Batch No. 0010006362). Additionally, two compounds, Dapagliflozin (Batch No. VL/S-DF-112/E) and Dapagliflozin D5 (Batch No.

VL/D-479/B), were obtained from Vivan Life Sciences. These materials were sourced from various manufacturers and used in the experimental setup for the intended analysis.

Various equipment was utilized in the analysis, including a pH Meter from Thermo Scientific, a Microbalance from Sartorius, an Ultra Sonicator and Nitrogen Evaporator from Athena, a Centrifuge from Eppendorf, a Refrigerator from Blue Star, a Deep Freezer (-70°C) from Panasonic, and a Hot Air Oven from Oswald. Additionally, a Column (Exsil Mono® 100 C18 100 Aº, 100*4.6mm, 3 µm) was employed, alongside a Multitube Vortexer from Labtop, an LC-MS/MS-4000 from Sciex, a Vortexer from Spinix, and Micropipettes in varying ranges (10-100µL, 20-200 µL, and 100-1000 µL) from Microlit, along with a Multipipette from Brand.

METHOD DEVELOPMENT PARAMETERS

ASCOT experiments are crucial during Method Development and Validation, aiming to detect carryover, which is the appearance of an analyte in a sample from a preceding one. The experiment involves injecting freshly spiked or bulk-spiked prepared samples with specific sample names like ASCOT Blank, ASCOT_LLOQ, and ASCOT_ULOQ. The acceptance criteria mandate that the carryover observed at the retention time of the analyte and internal standard in matrix blank injections should be less than 20% and 5% of the area response obtained in the LLOQ sample, respectively[FDA. 2018, ICH. 2011, Swartz, M. (2008), Internal SOPs]

The selectivity of a method is crucial for accurately determining specific compounds in analyzed matrices without interference from matrix components. To assess selectivity, 10 different lots of human blank plasma are randomly selected, including 6 normal, 2 lipemic, and 2 hemolyzed plasma lots. Matrix blank samples are prepared from each of these biological matrices, along with replicates of LLOQ samples, calibration standards, and bracketing QC samples from a single lot of matrix blank. All 10 blank samples and replicates are processed together with calibration standards and bracketing QC samples in a single analytical run. Interference at the retention time (RT) of the analyte and internal standard is evaluated by comparing interfering peak area responses in matrix blank samples to the mean area responses of the analyte and internal standard obtained in extracted LLOQ samples. Acceptance criteria include ensuring that the area response of interfering peaks at the analyte's RT in matrix blank samples is <20% of the mean analyte peak area response of LLOQ samples, and the area response of interfering peaks at the internal standard's RT is <5% of the internal standard's mean peak area response of LLOQ samples. Additional criteria involve meeting signal-to-noise ratio requirements, percentage of LLOQ and calibration standards within specific concentration ranges, coefficient of variation for LLOQ back-calculated concentration, and percentage of bracketing QC samples within ±15% of their nominal concentrations.[FDA. 2018, ICH. 2011, Swartz, M. (2008), Internal SOPs]

Precision refers to the consistency or reproducibility of measurements obtained from multiple samplings of the same homogeneous sample under specified conditions, while accuracy indicates how close these measurements are to the true or known value under the same conditions. Method development involves precision and accuracy batches, comprising matrix blank, zero standard (CS0), a set of calibration curve standards, and six samples each of quality control (QC) samples, including LLOQQC, LQC, MQC, and HQC. Freshly spiked batches are conducted using a pooled matrix for spiking, with the option to include additional QC (M1QC) if necessary. Acceptance criteria include ensuring that at least 67% of QC samples are within 15% of their respective nominal concentrations, except for LLOQQC which should be within 20%. Additionally, at least 50% of QC samples at each level should meet this criterion, with coefficients of variation (C.V.) for QC samples ≤ 15%, or ≤ 20% for LLOQQC. The mean percentage nominal concentrations of analyzed QC samples should be within ±15%, except for LLOQQC where it should be within ±20%.[FDA. 2018, ICH. 2011, Swartz, M. (2008), Internal SOPs]

Matrix effect refers to the direct or indirect alteration or interference in response caused by unintended analytes or other interfering substances in a sample .To assess the matrix effect ,measurements should be conducted in a minimum of 10 different lots of biological matrix ,including normal ,hemolyzed ,and lipemic lots for plasma ,or normal and lipemic lots for whole blood and serum .Low and high -quality control (LQC and HQC) samples are prepared in triplicate from each lot of matrix according to applicable standard operating procedures . These samples are then processed alongside freshly prepared calibration standards and duplicate bracketing QC samples for analysis .Acceptance criteria include ensuring that at least 67% of QC samples are within 15% of their respective nominal concentrations ,with a minimum of 50% meeting this criterion at each level . Additionally ,the coefficient of variation for QC samples at each level should be ≤15% and the mean percentage nominal concentrations of analyzed QC samples should fall within 100 ±15% .FDA .2018 ,ICH .2011 ,Swartz ,M . (2008) Internal SOPs]

Recovery in analytical processes denotes the extraction efficiency ,expressed as a percentage of the known amount of an analyte recovered through sample extraction and processing .To determine recovery ,the detector response from extracted quality control (QC)samples is compared to that of corresponding post -spiked QC samples .Recovery assessments are conducted for low ,medium ,and high -quality control samples ,with six samples prepared and processed for each level according to the extraction procedure . Additionally ,18 blank samples are extracted using pooled plasma or an accepted blank matrix .Post -extracted recovery samples are prepared by spiking extracted blank matrix with the analyte and internal standard working solutions .Injected samples ,both extracted and post -extracted ,are analyzed in six replicates at each QC level ,with the average response compared between the two sets . Internal standard recovery is determined

independently at the concentration level specified in the analytical method .Acceptance criteria include recovery close to 100% ,although values may be lower ,provided they are consistent , precise , and reproducible . Investigation and justification are required if recovery exceeds 100% .The coefficient of variation for mean recovery and area response of analyte should be ≤15% ,ensuring method reliability and accuracy . FDA .2018 ,ICH . 2011 ,Swartz ,M . (2008) In,nternal SOPs]

Bench -top stability assessment for spiked samples evaluates the stability of analytes in a biological matrix during the expected time the samples are kept on the bench during processing . Six low -quality control (LQC) and high -quality control (HQC) samples are retrieved from a deep freezer and placed on the bench for at least 6 00 hours or the specified duration mentioned in the method standard operating procedure (SOP) After this period ,all stability samples ,along with freshly prepared calibration curve standards , quality control samples , and six aliquots each of LQC and HQC , are processed and analyzed in a single run or batch .The bench -top stability duration is calculated based on the difference between the times when the QC samples were placed on the bench and the start of processing .Mean percentage nominal concentrations are calculated using a specific formula .Acceptance criteria include ensuring that at least 67% of comparison and stability QC samples are within ±15% of their nominal concentrations ,with a minimum of 50% meeting this criterion at each level .Additionally ,the percentage change should not exceed ±15% when comparing the mean back -calculated values of stability samples with their nominal concentrations and comparison samples .Mean percentage nominal concentrations should fall within ±15% at each level ,and the coefficient of variation at each level should be ≤15% ,ensuring method reliability and accuracy . FDA .2018 ,ICH .2011 ,Swartz ,M . (2008) In,nternal SOPs]

METHODOLOGY

Stock Solution Preparation:

To prepare the stock solution of Dapagliflozin (CC) ,approximately 2 mg of Dapagliflozin Working standard is weighed and transferred into a 2 000 ml volumetric flask .It is dissolved in 200 µl of methanol, and the volume is made up to the mark with methanol, resulting in a solution with a concentration of about 1 mg/ml of Dapagliflozin. The Dapagliflozin Working solution for CC is then prepared within a concentration range of approximately 0.025 µg/ml to 7.477 µg/ml using a diluent solution. Matrix-spiked calibration curve standards are next prepared within the range of approximately 1.006 ng/ml to 299.099 ng/ml. For the preparation of Dapagliflozin (QC) stock solution, about 2 mg of Dapagliflozin Working standard is weighed and transferred into a 2.000 ml volumetric flask, dissolved in 200 µl of methanol, and made up to the mark with methanol to achieve a concentration of about 1 mg/ml. Subsequently, the Dapagliflozin QC working solution is

prepared within a concentration range of approximately 0.025 µg/ml to 0.5875 µg/ml using a diluent solution. Matrix-spiked Quality Control samples are then prepared within the range of approximately 1.019 ng/mL to 235.006 ng/ml. Additionally, the Internal Standard Stock solution of Dapagliflozin D5 involves weighing about 1 mg of Dapagliflozin D5 Internal standard and transferring it into a 1.000 ml volumetric flask. It is dissolved in 200 µl of Methanol, and the volume is made up to the mark with methanol to achieve a concentration of about 1 mg/ml of Dapagliflozin D5. Lastly, the Dapagliflozin D5 Internal Standard dilution is prepared at a concentration of approximately 345.185 ng/ml using the diluent solution.[FDA. 2018, ICH. 2011, Swartz, M. (2008), Internal SOPs]

Extraction Procedure:

Upon retrieval from the deep freezer, the Calibration Curve standards, Quality Control samples, and subject samples are allowed to thaw at room temperature. Following thawing, the samples are vortexed to ensure thorough mixing. Next, 500 µl of Plasma samples are aliquoted into pre-labeled Ria vials. Fresh Working Aliquot, consisting of 672 µl of Blank Plasma with 28µl of Analyte working solution of CC/QC (excluding Blank and STD 0, which receive 28 µl of diluent), are prepared and vortexed for complete mixing, according to the extraction procedure's aliquoting volume. Subsequently, 50 µl of internal standard working solution (Dapagliflozin D5 at approximately 345.185ng/ml) are added to each pre-labeled Ria vial, with blank samples receiving 50 µl of diluent solution, followed by vortexing. Ethyl acetate (3.000 ml) is added to all samples and capped, then vortexed on a Multitube Vortexer for 10 minutes at 2500 rpm. The samples are then centrifuged at 4.0℃ and 4000 rpm for 5 minutes in a refrigerated centrifuge, and 2.400 ml of supernatant is separated into pre-labeled RIA vials. Following this, the samples are dried in a Nitrogen Evaporator at 40°C until dryness. Reconstitution of all samples with 0.250 ml of Mobile phase follows, with a brief vortexing, before transferring the samples to their respective pre-labeled Auto injector Vials.[FDA. 2018, ICH. 2011, Swartz, M. (2008)]

Preparation Of Solutions:

A 5mM Ammonium acetate solution is prepared by weighing approximately 0.3854 g of Ammonium acetate and transferring it into a 1000 ml volumetric flask. Then, 500 ml of HPLC-grade water is added, and mixed well to dissolve the compound, and the volume is made up to 1000 ml with HPLC-grade water. For the Mobile Phase Preparation, Acetonitrile and 5mM Ammonium acetate Solution are combined in a ratio of 75:25 v/v. Specifically, 750 ml of Acetonitrile and 250 ml of 5mM Ammonium acetate Solution are measured using a measuring cylinder, transferred into a 1000 ml reagent bottle, mixed thoroughly, and degassed in a Sonicator. The Diluent Solution, consisting of Methanol and water in a ratio of 50:50 v/v, is prepared by taking 250 ml of methanol and 250 ml of ultra-pure water,

transferring them into a 500 ml reagent bottle, mixing well, degassing in a Sonicator, and storing at room temperature. Lastly, the Rinsing Solution, with a composition of Methanol and water in a ratio of 70:30 v/v, is made by combining 700 ml of Methanol and 300 ml of ultra-pure water in a 1000 ml reagent bottle, mixing thoroughly, degassing in a Sonicator, and keeping at room temperature.[Skoog, Douglas A., *et al* 2017, Snyder, Lloyd R., *et al* 2010]
The chromatographic conditions utilized in the LCMS/MS 4000 instrument were as follows: Negative mode was employed, with an Exsil Mono® 100 C18 column measuring 100 A°, 100*4.6mm, and 3 µm. The mobile phase consisted of Acetonitrile: 5mM ammonium Acetate in a ratio of 75:25 v/v. The injection volume was set at 20 µl, and the flow rate was maintained at 0.400 ml/minute. The column oven temperature was maintained at 40 ℃, while the sample cooler temperature was set to 10 ℃. Expected retention times (RT) were approximately 2.89 minutes (±0.5 minutes) for Dapagliflozin and 2.87 minutes (±0.5 minutes) for Dapagliflozin D5, with a total run time of 4.00 minutes. Q1 and Q3 resolutions were set to unit. Detector conditions for the LCMS/MS 4000 included the utilization of Turbo Spray ion source in negative polarity, with CUR at 30.00, CAD at 6.00, IS at -4500.0, TEM at 550 0C, GS1 at 40.00, and GS2 at 60.00. Compound parameters for the LCMS/MS 4000 were as follows: DAP had a Q1 Mass of 467.300 and Q3 Mass of 329.100, while DAP-D5 had Q1 Mass of 472.200 and Q3 Mass of 334.150. Dwell time was set to 300.00 (m/sec), with DP at -30.0, EP at -10.0, CE at -25.0, and CXP at -20.0.[Skoog, Douglas A., *et al* 2017, Snyder, Lloyd R., *et al* 2010,25]

RESULTS AND DISCUSSION

Precision And Accuracy:

Table No 3: Precision And Accuracy

Sr. No	Sample Name	Analyte Peak Area(counts)	Analyte Retention Time(min)	ISPeak Area(counts)	IS Retention Time(min)	Accuracy (%)
1.	Blank	0	0.00	0	0.00	NA
2.	CS0	0	0.00	48942	3.04	NA
3.	CS1	522	3.06	48402	3.04	98.493
4.	CS2	819	3.05	28917	3.04	103.220
5.	CS3	15553	3.05	44991	3.04	97.142
6.	CS4	50790	3.05	59580	3.04	101.602
7.	CS5	115565	3.05	54147	3.04	98.862
8.	CS6	126476	3.05	40487	3.04	101.212
9.	CS7	174114	3.05	45471	3.04	98.634
10.	CS8	236868	3.05	48690	3.04	100.816
11.	Blank	0	0.00	0	0.00	NA
12.	LLOQQC-01	159	3.04	25869	3.04	69.198
13.	LLOQQC-02	615	3.05	48183	3.04	109.345
14.	LLOQQC-03	260	3.05	25499	3.04	93.800
15.	LLOQQC-04	377	3.05	30718	3.04	106.305
16.	LLOQQC-05	299	3.07	37045	3.04	80.756
17.	LLOQQC-06	350	3.07	37840	3.04	82.315

18.	LQC-01	1644	3.05	41510	3.04	98.318
19.	LQC-02	1705	3.05	42638	3.04	99.171
20.	LQC-03	1165	3.07	31617	3.06	92.287
21.	LQC-04	1786	3.05	42139	3.04	104.430
22.	LQC-05	1912	3.04	43466	3.04	107.927
23.	LQC-06	2070	3.05	48364	3.04	105.326
24.	M1QC-01	20362	3.05	40258	3.04	103.120
25.	M1QC-02	21249	3.04	43627	3.04	99.343
26.	M1QC-03	20776	3.04	42280	3.03	100.214
27.	M1QC-04	19427	3.05	38653	3.04	102.480
28.	M1QC-05	17824	3.05	36588	3.04	99.361
29.	M1QC-06	19788	3.04	39505	3.04	102.132
30.	MQC-01	84454	3.04	43148	3.04	101.189
31.	MQC-02	81422	3.05	41137	3.04	102.322
32.	MQC-03	73501	3.05	37339	3.04	101.189
33.	MQC-04	74367	3.05	37573	3.04	102.322
34.	MQC-05	65403	3.05	33996	3.04	99.461
35.	MQC-06	65014	3.05	32846	3.04	102.324
36.	HQC-01	137232	3.04	34740	3.04	104.214
37.	HQC-02	140378	3.05	34779	3.04	106.482
38.	HQC-03	137633	3.05	35960	3.04	100.978
39.	HQC-04	135861	3.05	34436	3.04	104.084
40.	HQC-05	133516	3.05	34567	3.04	101.904
41.	HQC-06	141561	3.05	35445	3.04	105.363

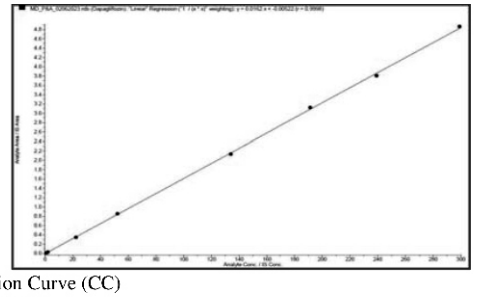
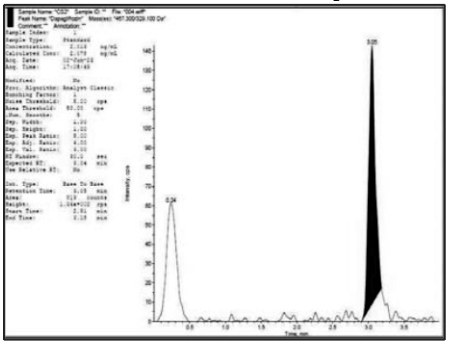
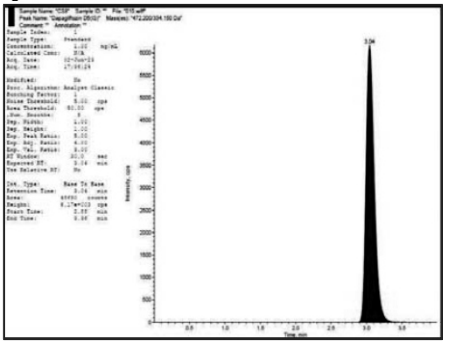


Figure No 2: Calibration Curve (CC)

CHROMATOGRAM

Figure No 3:CS1Figure No 4:CS8





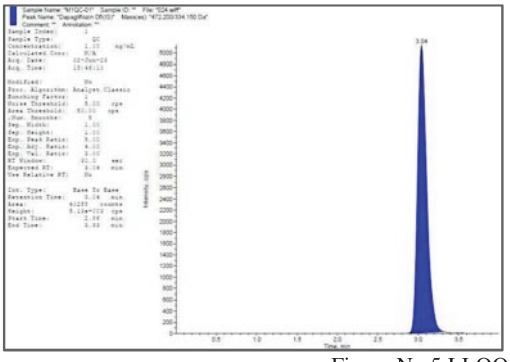
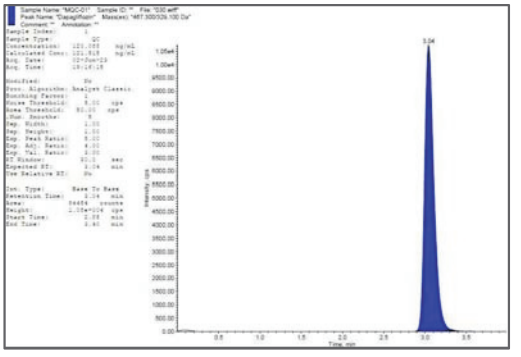



Figure No 5:LLOQQCFigure No 6:LQC

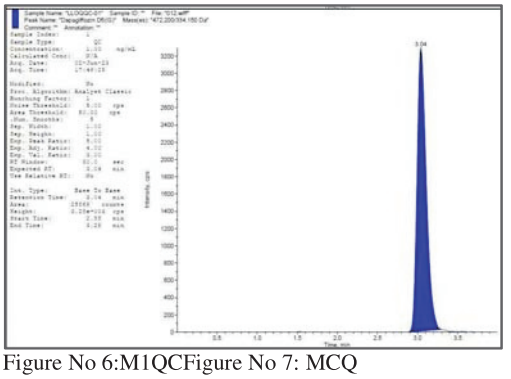
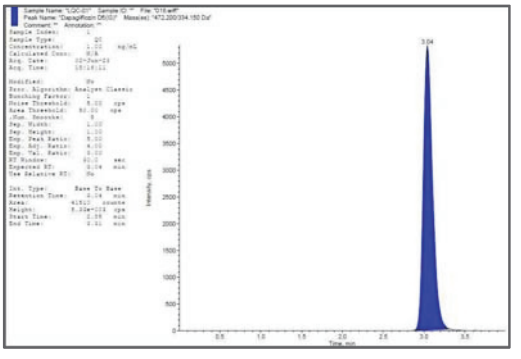



Figure No 6:M1QCFigure No 7: MCQ

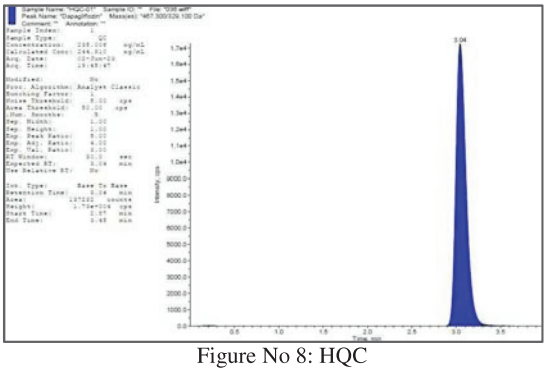


Figure No 8: HQC

SUMMARY

System Suitability: The % RSD was found to be 0.3, with a USP Tailing value of 1.1 and Theoretical Plates calculated as 14038.

Specificity: In terms of identification, no issues were observed. The retention time (R.T) of the Standard Solution was recorded as 3.191, while that of the Sample Solution was 3.4933.

No interference or peak purity issues were detected. Both the Standard and Sample Solutions exhibited specificity.

Precision:

Method Precision was determined to be 0.3%, while Intermediate Precision was slightly higher at 0.4%.

Accuracy:

At the 50% level, the accuracy was measured at 100.6%. Similarly, at the 100% level, the accuracy was recorded as 100.4%, and at the 150% level, it was 100.7%.

Linearity: The Correlation Coefficient (R) was calculated as 0.9993, indicating strong linearity. The %Y-intercept was found to be 0.31%.

CONCLUSION

The developed Bioanalytical Method was found to be rapid, sensitive, simple, specific, accurate, precise, and cost-effective for extraction of Dapagliflozin from Human Plasma using K3EDTA Anticoagulant. This method was employed inthe present investigation for extraction of Dapagliflozin using LCMS-MS 4000 with column Exsil Mono® 100 C18 100 Aº 100*4.6mm 3 µm, flow rate 0.4 ml per min with injection volume 20µl by using software analyst (version 1.6.3).The working solution of Dapagliflozin was prepared in diluent (Methanol: Water::50:50). Different injection volumes and different pure solvents of varying polarity in different proportions as mobile phase for the development of chromatogram. The Mobile phase that was found to be more suitable was Acetonitrile and 5mM Ammonium Acetate in a ratio of 75:25, with this mobile phase the chromatogram was in proper peak size. This selected chromatographic condition has good resolution and optimum retention time with good response. These practically taken parameter results from LCMS-MS indicate that the developed method can be successfully applied for the estimation of Dapagliflozin in human plasma with K3EDTA Anticoagulant in tablet dosage form.

REFERENCES

American Diabetes Association (2020). 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes—2020. *Diabetes Care*. 43(Supplement 1): S98-S110.

Amidon GL, Lennernäs H, Shah VP, Crison JR (1995). A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability. *Pharmaceutical Research*. 12(3): 413-420.

Bailey CJ, Gross JL, Pieters A, Bastien A, List JF (2010). Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with metformin: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 375(9733): 2223-2233.

Bailey CJ, Iqbal N (2011). Renal glucose reabsorption inhibitors to treat diabetes. *Trends in Pharmacological Sciences*. 32(2): 63-71.

Barnett AH, Mithal A, Manassie J, (2014). Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *New England Journal of Medicine*. 373(22): 2117-2128.

Blanch, Harvey W., and Carlos R. Soccol, eds (2011). Comprehensive biotechnology. Elsevier.

Chai, Xin-Sheng (2009). Sample preparation in chromatography. *Journal of Separation Science*. 32(6): 893-894.

Dapagliflozin (Farxiga) (2021). In Lexicomp Online. Wolters Kluwer Health, Inc. Retrieved from https://online.lexi.com

Davit BM, Nwakama PE, Buehler GJ, *et al* (2009). Comparing generic and innovator drugs: a review of 12 years of bioequivalence data from the United States Food and Drug Administration. *Ann Pharmacother*. 43(10): 1583-1597.

European Medicines Agency (2011). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**.

Farxiga (dapagliflozin) [Prescribing Information] (2021). AstraZeneca Pharmaceuticals LP. Available from: https://www.azpicentral.com/farxiga/farxiga.pdf#page≤1

FDA approves Farxiga for heart failure with reduced ejection fraction (2021, May 5). U.S. Food and Drug Administration. https://www.fda.gov/drugs/drug-safety-and-availability/fda-approves-farxiga-heart-failure-reduced-ejection-fraction

Ferrannini E, Ramos SJ, Salsali A, Tang W, List JF (2010). Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. *Diabetes Care*. 33(10): 2217-2224.

Internal SOPs from a relevant laboratory or institution.

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Validation of Analytical Procedures: Text and Methodology (ICH Q2(R1)). FDA. 2018

Kole, Prashant Laxman, (2011). Solid phase extraction: trends, opportunities and recent advances. *Journal of the Brazilian Chemical Society*. 22(4): 617-630.

Neal B, Perkovic V, Mahaffey KW, (2017). Canagliflozin and cardiovascular and renal events in type 2 diabetes. New England Journal of Medicine. 377(7): 644-657.

Poole, Colin F., and Michael Cooke (2012). Liquid-liquid extraction. Liquid-phase extraction. Elsevier: 53-116.

Scheen AJ (2014). Pharmacodynamics, efficacy and safety of sodium-glucose co-transporter type 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus. *Drugs*. 74(12): 1411-1428.

Shah VP, Midha KK, Dighe S, (1991). Analytical methods validation: bioavailability,

bioequivalence and pharmacokinetic studies. Conference report. *European Journal of Drug Metabolism and Pharmacokinetics*. 16(4): 249-255.

Skoog, Douglas A., (2017). High-performance liquid chromatography. Principles of instrumental analysis. *Cengage Learning*: 800-809.

Snyder, Lloyd R., *et al* (2010). Introduction to modern liquid chromatography. Wiley.

Swartz, M. (2008). Handbook of Analytical Validation.

Tischer, Thomas, and Wolfgang Weder (2010). The shear behaviour of protein precipitates and its impact on process design. *Chemical Engineering Science*. 65(16): 4762-4768.

U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM) (2018). Guidance for Industry: Bioanalytical Method Validation. Accessed January 10, 2022. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry

U.S. Food and Drug Administration (FDA). Guidance for Industry: Bioanalytical Method Validation. FDA. 2018

Viswanathan CT, Bansal S, Booth B, *et al* (2007). Workshop/conference report--Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *AAPS J*. 9(1): E30-E42.

Wilding JP, Woo V, Soler NG, *et al* (2013). Long-term efficacy of dapagliflozin in patients with type 2 diabetes mellitus receiving high doses of insulin: a randomized trial. *Annals of Internal Medicine.* 159(6): 405-415.

Zhang, Wenda, and Wei Ning Chen (2015). Protein precipitation: Principles and applications. Protein precipitation: Principles and applications. Springer, New York, NY: 1-10.

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STUDY OF ECOLOGY OF ANURANS (TOAD/FROG) IN RAJASTHAN

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The diversity of reproductive and life-history tactics exhibited by amphibians is astounding, encompassing a wide range of nest-building techniques and behaviors. Nesting behavior in this cladebroadly defined as a site chosen or constructed for eggs and youngis closely tied to the amphibious lifestyle of this group, even though anuran amphibians (frogs and toads) are not recognized for having nests. Anurans include frogs and toads. Out of the three amphibian species that are now recognized, anurans are the most varied and widely distributed. All around the world, anurans are present. Cities and gardens are common places to see frogs. Anuran diversity is highest in tropical climates; however, it is uncommon to find anuran species in polar regions, some marine islands, and very xeric deserts.

Rajasthan state is the largest state in the nation, covering an area of 3.42 lakh square kilometers. It is divided into seven administrative divisions, each with 33 districts. Frogs and toads are abundant species in Rajasthan, which is approximately 1,32,077 square miles. The western three-fifths of Rajasthan comprise the immense Indian Desert. Until recently, nothing was known about the fauna in Rajasthan's Abu Hills. There have been reports of physiological and climatic changes in the desert recently. Therefore, it is now essential to examine it from every angle. Over the past few years, different Zoological assessment of India parties have conducted a comprehensive assessment of the area.

INTRODUCTION

Amphibians are very important to the environment and the economy, primarily as anurans. Amphibians play a useful function in preventing pest damage to crops and vegetables and are formidable predators of several agricultural pests and disease-transmitting insects. Additionally, amphibians serve as excellent bioindicators of environmental deterioration. Frogs and toads are examples of tailless amphibians belonging to the Anura order of class amphibia. With a global distribution spanning around 5,400 species, the order Anura, also known as Salientia, is well-known and mostly valued for its nutritional and therapeutic properties. The four families that make up the order Anura Bufonidae,

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Dicroglossidae, Megophryidae, and Microhylidae are the only ones that still exist among the amphibian class. Together, these four families comprise 12 genera and 21 species. (Khan, S.N. 2004.)

Amphibians display a remarkable range of reproductive and life-history tactics, encompassing diverse methods of nest building and nest-related behaviors. Even though frogs and toads are not recognized for having nests, the clade's nesting behavior which is generally described as a site selected or built for eggs and young is closely associated with the amphibious lifestyle of this group. Anurans' reproductive diversity has been fueled by their shift to a more terrestrial lifestyle, which has resulted in the repeated, independent evolution of nests and nesting behaviors. The preservation of an aquatic habitat for developing young is, in fact, a fundamental characteristic of several noteworthy anuran adaptations, including nesting behavior. Anurans' morphological, physiological, and behavioral diversity are closely linked to their expanding terrestrial reproduction, which opens up new avenues for research into the evolutionary ecology of nests, their builders, and their contents.

There are 5918 amphibian species in the globe according to the Global Amphibian Assessment (GAA), which was published in June 2007. With 88% of the total, or 5211 different species, frogs and toads top the list. Thirty-nine, or 1590, of them have the classification of vulnerable, endangered, or critically endangered. 311 species of amphibians are known to exist in India. Rajasthan is home to several types of amphibians. Mansukhani reported eight species of frogs, whereas Kumar studied with amphibians of Sitamata WLS. While Sharma has identified 12 species of amphibians from around Rajasthan, Sharma and Sharma *et al*. have focused on various features of amphibians in Rajasthan.This current study focuses on the frogs and toads that can be found along the Rajasthani portion of the Chambal River from Kota to Dholpur. (Sharma, S. K. 2005).

Habit and Habitat

The toad is a cold-blooded, poikilothermous, or exothermic animal, meaning that its body temperature is determined by the outside temperature. In the colder months of the year, toads go through a stage known as winter sleep or hibernation. Although an adult toad prefers to dwell on land, it may spend some time submerged in water if needed. In both the biological and literal senses-amph ≤ both, bios ≤ life-it is unquestionably an amphibian. The toad is perfectly content both on land and in the water. The toad must always stay in damp, dark areas when on land, ideally close to water. Since the skin of a toad is an auxiliary respiratory organ, it is naturally kept moist. It seeks to stay out of the sunlight.(Altig, R. 2007)

The toad is a predatory animal that uses its sticky mouth to collect insects. Being nocturnal creatures, toads typically emerge from their hides at twilight. Especially during breeding season, the males' vocal sac helps them make a croaking sound. Toads breed in water, although they live on land. With the exception of the reproductive system, every organ system in a toad is adapted to life on land. Reproduction required it to return to its ancestral

watery home.

MATERIALS AND METHODS

STUDY AREA

Multiple methods of collection have been developed for amphibians due to their frequent occurrence in both aquatic and terrestrial environments. The most straight forward method for gathering. While they were in suburban water reservoirs or ponds at crops, frogs were collected using a dip net as a specimen collection technique. When they were in scrub land, they were slapped with a hand. When it came to gathering specimens from water bodies devoid of vegetation or those with dense vegetation, a variety of traps, such as the funnel trap and pitfall trap, were more useful than sewing for gathering both adult amphibians and their larvae. Additionally useful for gathering amphibians on dry land were the funnel trap and pitfall trap. (Bossuyt, F. and Dubois, 2001).

The Chambal River travels 225 miles in a north-easterly direction across Rajasthan. There are 217 km of additional Chambal flow between Madhya Pradesh and Rajasthan, and an additional 145 km between Madhya Pradesh and Uttar Pradesh. The 600 km long National Chambal Sanctuary is a tri-state area that is protected along the Chambal River between the Kota Barrage and the Chambal-Yamuna confluence.(Daniels, R.J.R. 2005).

The National Chambal Sanctuary is located between 75°34' and 79°18' E and 24°55' to 26°50' N. It is made up of the large arc that the Chambal describes, which runs between the Chambal-Yamuna confluence in Uttar Pradesh and the Jawahar Sagar Dam in Rajasthan. Two sections of the Chambal River are designated as National Chambal Sanctuary along this arc: the lower segment, which extends from Keshoraipatan in Rajasthan to the Chambal-Yamuna confluence in Uttar Pradesh, and the upper segment, which runs from Jawahar Sagar Dam to Kota Barrage.18 point locations under the river in the district(s) of Chittorgarh, Kota, Sawai-madhopur, Karauli and Dholpur were surveyed to determine the variety of anuran fauna.

Reproduction

Breeding behaviour

Among the most distinguishing characteristics of the Anura are its breeding habits. The majority of frogs lay their eggs in bodies of fresh water because they can only develop in moist conditions. Large populations of many species gather for brief breeding seasons at makeshift pools. While some live year-round, others breed among the mountain streams. Breeding individuals are not concentrated in one location for either of the latter two species or those that breed on land. The ladies are drawn to the breeding place by the male's mating call in all situations. The females' ability to distinguish between mating calls belonging to their

own species and those of other species has been noted in both the field and the lab.19 April 2021).

Specific calling site distinctions between males help the frogs keep their identities at communal breeding locations like ponds, swamps, or streams. The primary means of premating isolation, however, that keeps closely related species from hybridizing when they live in the same region and breed at the same time and location is differences in mating sounds. With two openings on the floor of the mouth that open into a vocal pouch, most species of frogs have very basic voice cords. Vocal cords vibrate when air is pumped from the lungs over them, producing a certain pitch and pulse in the sound.

The vocal pouch is filled with air and functions as a resonating chamber to emphasize either the same frequency or one of its harmonics. This is how different species of frogs make their distinct cries. Although most frogs are thought to be peaceful creatures, recent research has revealed that certain species can be violent, particularly during the mating season. Male green frogs (Rana clamitans) and bullfrogs (Lithobates catesbeianus) use biting, bumping, and kicking to keep other males out of their calling regions. Hyla faber, a South American hylid known for making nests, has a long, pointed spine on its thumb that males use to injure one another during wrestling. From the leaves of herbaceous plants, the little Dendrobates pumilio of Central America cries.

When one calling male invades another's area, it starts a wrestling match that ends when one of the males gets thrown off the leaf. Colostethus inguinalis, a dendrobatid species found in Central America, with male calling locations located on stones in streams. A male intruder causes the resident to issue a territorial call. If the intruder persists, the resident charges him in an attempt to butt him off the boulder. In streambeds, female members of the Venezuelan C. trinitatus wrestle to defend their territories.

Females seek out and approach guys who call to them. The female chooses the location for the egg deposit after the male has embraced her in a copulatory gesture known as amplexus. The male of the more primitive frogs (the families Ascaphidae, Leiopelmatidae, Bombinatoridae, and Discoglossidae as well as the mesobatrachians) grasps the female from above and around the waist (inguinal amplexus), whereas the more advanced frogs (the neobatrachians) move the position anteriorly to the armpits (axillary amplexus). The latter arrangement likely promotes more effective fertilization by bringing the amplectic pair's cloacae closer together.

Egg laying and hatching

Either as solitary eggs, surface films, strings, or clumps, the majority of frogs lay their eggs in calm water. Attached to sticks or submerged foliage, or freely suspended in the water are other options for the eggs. In streams where the eggs are not affected by the current, certain frogs deposit their eggs. These eggs are usually securely affixed to the lee sides or

undersides of rocks. More eggs are presumably produced than by any other anuran species, including the big pond-breeding frogs of the genus Rana and the toads of the genus Bufo. The North American bullfrog, Leporida catesbeianus, is known to lay up to 10,000 eggs in a single clutch. (19 April 2021).

Eggs can develop in the most oxygenated area of the pool by extending their habit of forming a film on the water's surface, which is likely an adaptation for oviposition in shallow temporary pools. A number of the Hylidae family of tree frogs found in the American tropics exhibit this type of egg deposition; Smilisca baudinii, one of these species, is known to lay over 3,000 eggs annually. There are typically only 200 eggs laid by frogs that breed in the gushing mountain streams. (Chanda, S. K. 2002).

Numerous changes have been made to address the issue of egg fertilization in quickly moving water. Long cloacal tubes allow certain stream-breeding hylids to direct semen onto the developing eggs. Huge testes on several other hylids appear to produce enormous amounts of sperm, which aids in ensuring conception. The cloaca of the male Ascaphus truei tailed frog serves as an extension for copulatory purposes, allowing the male to insert sperm into the cloaca of the female.

RESULTS AND DISCUSSION

Among the members of the dicroglossidae family were Rana hexadactyla, Rana cyanophlyctis, Rana limnocharis, Rana tigrina, Rana breviceps, and Rana rolandae. Among the members of the family Bufonidae were Bufo andersoni Boulenger and Bufo melanositctus Schneider. Microphyla ornata and Uperdon systoma were members of the family Microhylidae. Polypedates maculates belonged to the family Rhacophoridae. (Agarwal, S. K. (1978).

A) Rana hexadactylaLesson
Rana hexadactyla lesson is also known as euphlyctis hexadactylus, green skin Frog or five finger frogs.
Kingdom : animalia
Phylum : chordate
Class : amphibua
Order : anura
Family : dicroglossidae
Genus: rana
Species: hexadactylus

Distribution
The Rana hexadactyla lessonis mostly found in jaipur districts.

Rana hexadactyla Lesson

It has thin fingers, the first reaching slightly past the second, webbed toes, and a fourth toe that isn't much longer than the third or fifth.
There are no external tubercles and tiny inside metatarsal tubercles.
Male voice vesicles on the outside.(Agarwal, S.K. and Niazi, I. A. (1977).
(Agarwal, S.K. and Niazi, I. A. (1977). Normal table of developmental stages of the Indian bull frog Rana tigerina. Proc. Nat. Acad. Sci. India, B2: 79-92.)

B) Rana cyanophlyctis Schneider
Rana cyanophlyctis Schneideris also known as Indian skipper frog or skittering frog.
Kingdom:animalia
Phylum: Chordate
Class: amphibua
Order: anura
Family : dicroglossidae
Genus : rana
Species : cyanophlyctis
Distribution Rana cyanophlyctis Schneideris mostly found in districts of Barmer, ganganagar, udaipur, sirohi, jaisalmer, jodhpur, nagaur, Pali,s ikar, ajmer, Jaipur.
Schneider is 52–61 mm long. They rarely appear outside of the water and are left in it. They can jump from a floating position straight out of the water. The fingers are sharp and thin. Head in the middle. Much thinner than the upper eyelid is the interorbital gap. Small tubercles subarticular. The skin is brown or olive in color above, darkly speckled or marbled, and seldom

Rana cyanophlyctis Schneideris
missing below, with two blackish streaks on the hinder side. Districts in Udaipur have reddish brown specimens, while districts in Nagaur have olive specimens. Skin featuring distinct rows of pores and tiny tubercles. (Bossuyt, F. and Dubois, A. (2001).
(Bossuyt, F. and Dubois, A. (2001). A review of the frog genus Philautus Gistel, 1848 (Amphibia, Anura, Ranidae, Rhacophorinae). Zeylanica, 6, 1-112.)

C) Rana limnocharis wiegmann

Rana limnocharis wiegmann is also known as crab eating frog or fejervarya frog.
Kingdom : Animalia
Phylum : Chordata
Class : amphibia
Order : anura
Family : dicroglossidae
Genus : Rana
Species : limnocharis
Distribution

Rana limnocharis wiegmann is mostly found in districts of jaipur, Udaipur, pali, nagaur, sirohi.

The greatest size of Rana limnocharis wiegmann is 24 mm. Tadpoles of the crab-eating frog can even survive in pure saltwater, and the species can flourish in brackish water. Their skin tone is dark brown.(Dutta,S.K.1997)

Rana limnocharis

(Dutta, S.K. 1997. Amphibians of India and Sri Lanka. Odyssey Publication House: 1-338.)
(Dutta, S. K. and Routray, N. 1990. First record of Rana hexadactyla (Anura: Ranidae) from Orissa, with some comments on their ecology and distribution. Herpeton, 3: 5-6.)

D) Ranatigrina Daudin
Rana tigrina daudin is also known as hoplobatrachus tigerinus.

Kingdom : Animalia
Phylum : Chordata
Class : Amphibua Order : Anura
Family : dicroglossidae
Genus : Rana
Species : tigerinus
Distribution
Rana tigrina tigrina daudin is mostly found in districts of nagaur, Udaipur, jaipur, ganganagar.

Rana tigrina

(Sharma, S.K. (1992). First record of Uperodon systoma from Rajasthan. J. Bombay Nat. Hist. Soc., 89 (1): 133-134.)
(Sharma, S.K. (1995 a). An overview of the amphibians and reptilian fauna of Rajasthan. Flora and Fauna, 1(1): 47-48.)

REFERENCES

Abraham, R. K., Mathew, J. K., Cyriac, V. P., Zachariah, A., Raju, D. & Zachariah, A. (2015) A novel third species of the Western Ghats endemic genus Ghatixalus (Anura: Rhacophoridae), with description of its tadpole. *Zootaxa,* 4048 (1): 101–113.

Agarwal, S. K. & Niazi, I. A. (1977) Normal table of developmental stages of the Indian bull frog Rana tigrina Daud. (Ranidae, Anura, Amphibia). The Proceedings of the national Academy of Sciences, India, (B2), 47: 79–92.

Agarwal, S.K. and Niazi, I. A. (1977). Normal table of developmental stages of the Indian bull frog Rana tigerina. *Proc. Nat. Acad. Sci. India,* B2: 79-92.

Agarwal, S. K. & Niazi, I. A. (1980) Development of mouthparts in the tadpoles of Rana tigrina Daud.

Proceedings of the Indian Academy of Sciences (Animal Sciences), 89 (2): 127–132.
Anand Padhye, Kelum Manamendra-Arachchi, Anslem de Silva, Sushil Dutta, Tej Kumar Shrestha, Sabitry Bordoloi, *et al*. Hoplobatrachus tigerinus. IUCN Red List of Threatened Species. Version International Union for Conservation of Nature. 2008, 2009, 2010.
Altig, R. (2007) A primer for the morphology of anuran tadpoles. Herpetological Conservation Biology,
Bhaduri, J. L. (1944a) Further locality records of Rana hexadactyla lesson in Bengal, with brief notes on its tadpoles. *Journal of the Bombay natural History Society*, 44 (3): 484–485.
Boulenger, G.A. (1890). In: The Fauna of British India Including Ceylon and Burma. Reptiles and Batrachia. Taylor and Francis, London, xviii + 541.
Bossuyt, F. and Dubois, A. (2001). Are view of the frog genus Philautus Gistel, 1848(Amphibia,Anura,Ranidae,Rhacophorinae).Zeylanica, 6, 1-112.
Chanda, S.K. (2002). In: Handbook - Indian Amphibians. Zoological Survey of India, Calcutta, viii + 335.
Chacko, T. (1965) The development and metamorphosis of the hyobranchial skeleton in Rana tigerina the Indian bull frog. Acta zoologica, 46: 311–328.
Department of Environment and Primary Industries. Asian black-spined toad, 2014. Victorian Government.http://www.depi.vic.gov.au/agriculture-andfood/pests-diseases-and-weeds/pest-animals/a-z-of-pestanimals/asian-black-spine-toad; retrieved 2017-05-28.
Clutton-Brock T. 1991. The evolution of parental care. Princeton, NJ: Princeton University Press.
Collias NE, Collias EC. (1984). Nest building and bird behavior. Princeton, NJ: Princeton University Press.
Das I. Folivory and seasonal changes in diet in Rana hexadactyla (Anura: Ranidae). Journal *of Zoology*. 1996; 238(4):785-794.
Dutta,S.K.1997.Amphibians of India and Sri Lanka.OdysseyPublicationHouse:1-338.
Daniels, R. J. R. (2005). Amphibians of peninsular India. Universities Press, Hyderabad, pp.268.
Khan, S.N. 2004. Eco-biological and developmental studies of Anuran (Amphibia) biodiversity of some selected fluoride rich areas of Rajasthan. Ph. D. Thesis, M.D.S. Univ., Ajmer (Rajasthan).
McCann, C. (1932).Noteson Indian batrachians. J. Bombay Nat. Hist. Soc.,36:152-180.
McCann, C. (1934). Occurrence of the Six-toed frog (Rana hexadactyala Lesson) in Bombay Presidency. *J. Bombay Nat. Hist. Soc*., 37: 742.
Ray,P. (1996).Amphibian diversity in and around Tehri Damsite, U. P. India. Zoos' Print, 11 (5) :6.
Royle N, Smiseth P, Köliker M. (2012). The evolution of parental care. Oxford, UK: Oxford University Press.

Royle N, Smiseth P, Köliker M. (2012). The evolution of parental care. Oxford, UK: Oxford University Press.

Sharma, K.K., Dube, P, Khan, S.N., Ghatak, S. and Bhobharia Y. (2004). "Amphibian Biodiversity of some specific ecological condition of Rajasthan and conservation strategies". Management of Aquatic resources for biodiversity maintenance and conservation, held in Jodhpur, Rajasthan (Abstract).

Saidapur SK, Girish S. (2001)Growth and Metamorphosis of Bufo melanostictus Tadpoles: Effects of Kinship and Density". *Journal of Herpetology.*; 35(2):249 -254