



CONJUNCTIVITIS EYE DISEASE DETECTION APPLICATION: IMAGE PROCESSING AND ESTABLISHING NOVEL DRUG LEADS FROM MEDICINAL PLANTS

Priyanka S. Sakhare, Neha S. Nilawad, Payal G. Satpute, Shalini Bakal, Krutanjali Patil and Preenon Bagchi

Institute of Bioscience and Technology, MGM University, Aurangabad, India

Email: priyasakhare7499@gmail.com

ABSTRACT:

Pink eye, also known as conjunctivitis, is an inflammation or irritation of the conjunctiva, which covers the white portion of the eye. Allergies or a bacterial or viral illness may be to blame. Contact with eye secretions from an infected person can spread conjunctivitis, which can be quite contagious. Redness, itchiness, and eye tears are symptoms. Additionally, it may cause crusting or discharge around the eyes. According to research, adenoviruses are among the most common causes of this eye condition.

One of the many areas in which image processing is used is in the medical industry. Humans can develop a variety of eye conditions. Therefore, early detection is essential, especially for fragile organs like the eye. One of these eye illnesses is keratoconus.

The term "image pre-processing" refers to operations on images at the most basic abstraction level. If entropy is used as a measure of information, these processes actually reduce the amount of information present in the image. Pre-processing aims to improve the picture data by reducing unwanted distortions or enhancing specific visual properties that are important for subsequent processing and analysis tasks.

Clinical routines like diagnosis, treatment, and monitoring face new obstacles as a result of the ever-growing amounts of patient data being collected in the form of medical images. Image mining is the process of looking through data to find important information. It is used in machine learning and image processing. Using image processing to detect diseases in medical photographs. The identification and categorization of diseases are based on the type of human organ and picture. It is possible to automate and/or support doctors with clinical diagnosis using image processing and machine learning techniques.

Further, 3d structure of the viral receptor, causing Conjunctivitis is modelled using homology modelling. As per studies it is seen that eyebright (*Euphrasia officinalis*) and chamomile (*Matricaria chamomilla*) are two medicinal herbs used in this disorder. Their phytochemicals are retrieved and HTS is performed. As per HTS it is seen that quercetin and caffeic acid docks best with the viral receptor and hence they can be used as novel drug leads for the disorder.

KEYWORDS:

Image processing, eye diseases, Noise Removal, Gaussian Blur, Image Sharpening, edge detection, homology modelling, ADME, HTS.



INTRODUCTION:

Pink eye (conjunctivitis) is an inflammation or infection of the transparent membrane (conjunctiva) that lines our eyelid and covers the white part of our eyeball [1]. The reddish or pink eye happens when the blood vessels in the membrane covering your eye gets inflamed, making them more visible [1, 2]. This is what causes the white color of our eyes to appear pink. In the conjunctivitis pink eye can be irritated [3]. Treatment can be help make less painful. We can cure this infection with early diagnosis so that, it cannot be spread [1, 2, 3].

Generally, Conjunctivitis is etiology of eye redness and discharge [2, 3]. It could be infectious or non-infectious [2]. Viral Conjunctivitis is a common cause, followed by bacterial conjunctivitis [2]. Mostly conjunctivitis is allergic. They can spread easily from person to person [4].

It can affect people of any age or demographics. More than 80% of all cases are diagnosed by non-eye care specialist such as primary care providers, paediatricians, and nurse practitioners [1, 2, 3, 4].

Image pre-processing

Image pre-processing methods are intended for image improvement for the needs of next processing of the image (generally object recognition). Image pre-processing is a fundamental step for noise removal and for enhancing the quality of an original image. The quality of raw image can be improved by removing unusual parts from the background of an image [5].

Pre-processing is a common name for operations with images at the lowest level of abstraction both input and output are intensity images. These iconic images are of the same kind as the original data captured by the sensor, with an intensity image usually represented by a matrix of image function values (brightness's). The aim of pre-processing is

an improvement of the image data that suppresses unwilling distortions or enhances some image features important for further processing, although geometric transformations of images (e.g. rotation, scaling, translation) are classified among pre-processing methods [6].

Image processing:

Digital images play a key role in our day-to-day life. Millions of digital images are uploaded in every single minute [7]. They are the numeric representation of a two-dimensional image, normally binary. The digital images can be used in various applications like medical imaging, video surveillance, criminal investigations, remote sensing etc. [6, 7, 8].

The automatic analysis of images and their signals through various image processing techniques is of higher importance nowadays [9, 10, 11]. Image processing and computer vision plays a vital role in any fields related to image enhancement, manipulation and analysis. Image processing is a method that performs the analysis and manipulation of digitized images, to improve the quality of image. Adaptability, recurrence and precision in the original data preservation, are the principal advantages of digital image processing methods [12].

Image processing is spreading in various fields. Image processing is a method which is commonly used to improve raw images which are received from various resources. It is a technique to transform an image into digital form and implement certain actions on it, in order to create an improved image or to abstract valuable information from it. It is a kind of signal dispensation where image is an input and output are also an image or features related with image [12, 13, 14].



To perform the medical image processing and disease detection, a sequence of image processing operations is required to improve quality of acquired image and to perform the detection. These processing stages are:

- a. Noise suppression
- b. Sharpening
- c. Contrast Enhancement
- d. Image Segmentation
- e. Feature extraction
- f. Statistical analysis
- g. Classification based on a classifier

These steps are helps in improving the quality of the image and algorithms used in these methods are depends upon that condition or situation [15, 16, 17]. Various image processing techniques used in automated recent diagnosis and analysis of various eye disease are Enhancement, Registration, Image Fusion, Image Segmentation, Feature extraction, Pattern Matching, Classification, Statistical measurements and Analysis [18, 19].

1.Noise Removal:

Image noise can be defined as appearance of undesired traces and variations in the brightness or colour of your image. Noise in an image is the presence of artifacts that do not originate from the original scene content [21, 22, 23].

Image processing is the use of algorithms to process digital images. This allows a much wider range of algorithms to be applied to the input data and avoid problems of noise and signal distortion. Image noise is random variation of brightness or colour information in images. The removal of noise from images is a major task in the field of image processing, because it affects the quality of the image and leads to the loss of some of its important information through the impact of noise on it. To remove the noise in the images, different image filtering techniques are used [24, 25].

2.Guassian Blur:

The Gaussian blur feature in image processing, also known as Gaussian smoothing, is the result of blurring an image by a Gaussian function. If one takes a photo in low light, and the resulting image will have a lot of noise and Gaussian blur can mute that noise [24, 25, 26].

3.Image Sharpening:

Image sharpening encompasses an enhancement technique that highlights the edges with its fine details. This is done by adding a signal proportional to the original image to a high-pass filtered version of the image. Thus the sharpening of image helps in highlighting the fine details that are present in the image and enhance the details that are blurred due to some reason like adding noise [24, 25, 26].

4. Edge Detection:

The image edge demonstration decreases the amount of information to be processed. This contains vital information about the object's shape in an image. This is a process to locate the edges that having good orientation. It is an essential tool of image segmentation as it transforms the original image into edge image with the help of operators. Hence, it is a well-known process for identifying the discontinuities in intensity values. In this process the input image is inputted is converted into gray scale image, then the edge detector detects and extracts the edges present within an image as output [24, 25, 26, 27].

In this project, we can help the doctor and notify the patient about this disease and its solution.

HTS:

High-throughput screening (HTS) is one of the newest techniques currently used in drug design experiments and may be applied in biological and chemical sciences. HTS techniques are now used in the pharmaceutical industry for screening of enormous numbers of compounds in the drug



development process. This process allows automated testing of large numbers of chemical and/or biological compounds for a specific biological target. Today, High-Throughput Screening (HTS) has become a standard and important method for discovering novel drugs in various pharmaceutical industries to quickly test the biological or biochemical activity of a large number of molecules, usually drugs. This accelerates target analysis, as large-scale compound libraries can quickly be screened in a cost-effective way.

This technique is used as starting point for medicinal chemical optimization during pharmacological probe or drug discovery and development. The greatest advantage of this technique over other diagnostic approaches is that it gives a complete view of the vital phytosanitary status of a phytocompound; also, the sequence data can be analysed by multiple end-users or may be re-analysed as databases are expanded.

In HTS we are performing Homology modelling of the target, selection of the template and their

screening using Lipinski's rule of five, further using docking we get the drug lead.

Medicinal plants & properties:

Eyebright herb (*Euphrasia officinalis*) has been used as traditional herbal medicine for minor eye ailments, redness and irritation in Europe and India for centuries. It grows best in poor soil and being a semi-parasitic plant, it gets some of its water and nutrients from the roots of nearby plants. It is also used in homeopathy, as form of natural medicine that uses extremely diluted substances for treatments (Fig. 1(a)).

German chamomile (*Matricaria chamomilla*) is one of the most popular traditional medicinal herbs traditionally used chamomile tea to relieve eye infections, blocked tear ducts, and conjunctivitis. It contains flavonoids, hence may be helpful to reduce inflammation. It is a famous medicinal plant distributed worldwide and is widely used in traditional medicine to treat all kinds of diseases, including infections, neuropsychiatric, respiratory, gastrointestinal, and liver disorders (Fig. 1(b)).

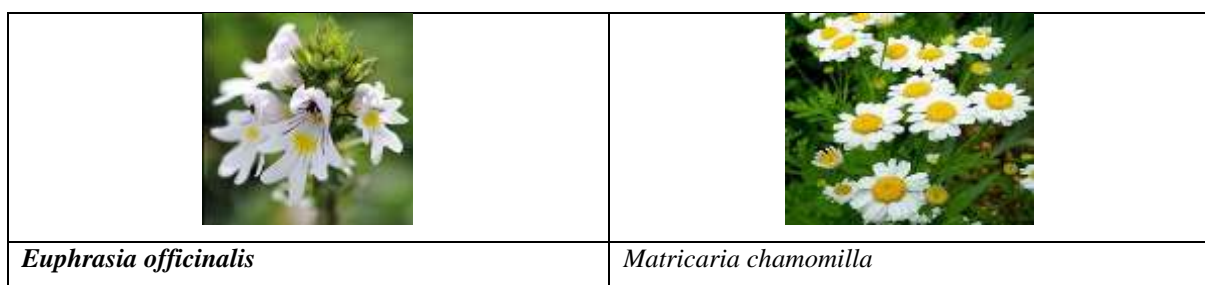
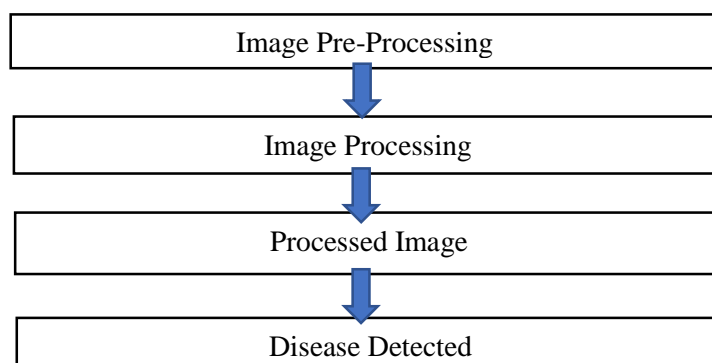
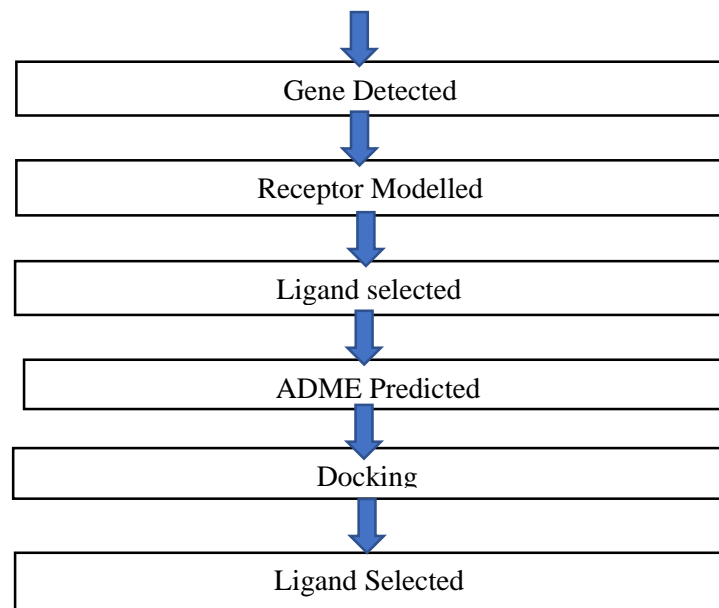


Fig. 1: Plants used

METHODOLOGY:





We used four different techniques for image processing

[https://opencv24-python-tutorials.readthedocs.io/en/latest/py_tutorials/py_tutorials.html]

1. Noise Removal:

Noise is generally considered to be a random variable with zero mean. Consider a noisy pixel, where I is the true value of pixel and N is the noise in that pixel. You can take large number of same pixels (say N) from different images and compute their average.

Ideally, you should get I since mean of noise is zero.

OpenCV provides four variations of this technique.

1. `cv2.fastNlMeansDenoising()` - works with a single grayscale image
2. `cv2.fastNlMeansDenoisingColored()` - works with a colour image.
3. `cv2.fastNlMeansDenoisingMulti()` - works with image sequence captured in short period of time (grayscale images)
4. `cv2.fastNlMeansDenoisingColoredMulti()` - same as above, but for colour images.

Common arguments are:

h : parameter deciding filter strength. Higher h value removes noise better, but removes details of image also. (10 is ok)

$hForColorComponents$: same as h , but for colour images only. (Normally same as h)

`templateWindowSize`: should be odd. (Recommended 7)

`searchWindowSize`: should be odd. (Recommended 21)

2. Gaussian Blur:

Image Blurring refers to making the image less clear or distinct. It is done with the help of various low pass filter kernels. In Gaussian Blur operation, the image is convolved with a Gaussian filter instead of the box filter. The Gaussian filter is a low-pass filter that removes the high-frequency components are reduced.

`Gaussian Blur (src, dst, ksize, sigmaX)`

This method accepts the following parameters –

`src` – A Mat object representing the source (input image) for this operation.

`dst` – A Mat object representing the destination (output image) for this operation.



ksize – A Size object representing the size of the kernel.

sigmaX – A variable of the type double representing the Gaussian kernel standard deviation in X direction.

3. Image Sharpening:

[<https://learnopencv.com/image-filtering-using-convolution-in-opencv/>]

The arguments to be passed in are as follows:

- src: This is the source image, i.e., the image that will undergo sharpening.
- ddepth: This is an integer value representing the expected depth of the output

image. We will set this value to be equal to -1. In doing this, we tell the compiler that the output image will have the same depth as the input image.

- kernel: This is the kernel we will use to conduct our desired process.

4.Edge Detection:

[<https://www.tutorialkart.com/opencv/python/image-edge-detection/>]

[<https://learnopencv.com/edge-detection-using-opencv/>]

Codes are shown by the following code:

```
from tkinter import * [28]
from tkinter import messagebox
from tkinter import ttk
import cv2
import numpy as np
# Create an instance of tkinter frame
win= Tk()
# Set the size of the tkinter window
win.geometry("700x400")
# Define a function to show the popup message
def show_msg():
    messagebox.showinfo("Message","Hey There! I hope you are doing well.")
def show_msg1():
    img_1 = cv2.imread('/home/student/PycharmProjects/ pythonproject2.py/venv/4.jpg')
    img_2 = cv2.fastNlMeansDenoisingColored(img_1, None, 10, 10, 7, 21)
    cv2.imshow('Original image',img_1)
    cv2.imshow('Noise Removal image', img_2)
    cv2.waitKey(0)
    cv2.destroyAllWindows()
def show_msg2():
    img_1 = cv2.imread('/home/student/PycharmProjects/ pythonproject2.py/venv/4.jpg')
    img_2 = cv2.GaussianBlur(img_1, (3, 3), 0)
    cv2.imshow('Original image',img_1)
    cv2.imshow('Guassian blur image', img_2)
    cv2.waitKey(0)
```



```
cv2.destroyAllWindows()
def show_msg3():
    img_1 = cv2.imread('/home/student/PycharmProjects/ pythonproject2.py/venv/4.jpg')
    kernel = np.array([[0, -1, 0],
                       [-1, 5, -1],
                       [0, -1, 0]])
    img_2 = cv2.filter2D(src=img_1, ddepth=-1, kernel=kernel)
img_2 = cv2.Canny(img_1, t_lower, t_upper)
cv2.imshow('Original image',img_1)
cv2.imshow('Edge Detection', img_2)
cv2.waitKey(0)
cv2.destroyAllWindows()
# Add an optional Label widget
Label(win, text= "Eye Disease Detection Image Processing Techniques", font= ('Aerial 17 bold
italic')).pack(pady= 30)
# Create a Button to display the message
ttk.Button(win, text= "Image noise removal", command=show_msg1).pack(pady= 20)
ttk.Button(win, text= "Image Gaussian Blur", command=show_msg2).pack(pady= 20)
ttk.Button(win, text= "Image Sharpening", command=show_msg3).pack(pady= 20)
ttk.Button(win, text= "Image edge detection", command=show_msg4).pack(pady= 20)
win.mainloop()
```

RESULTS:

The results of image processing are given in fig. 2.

Output:

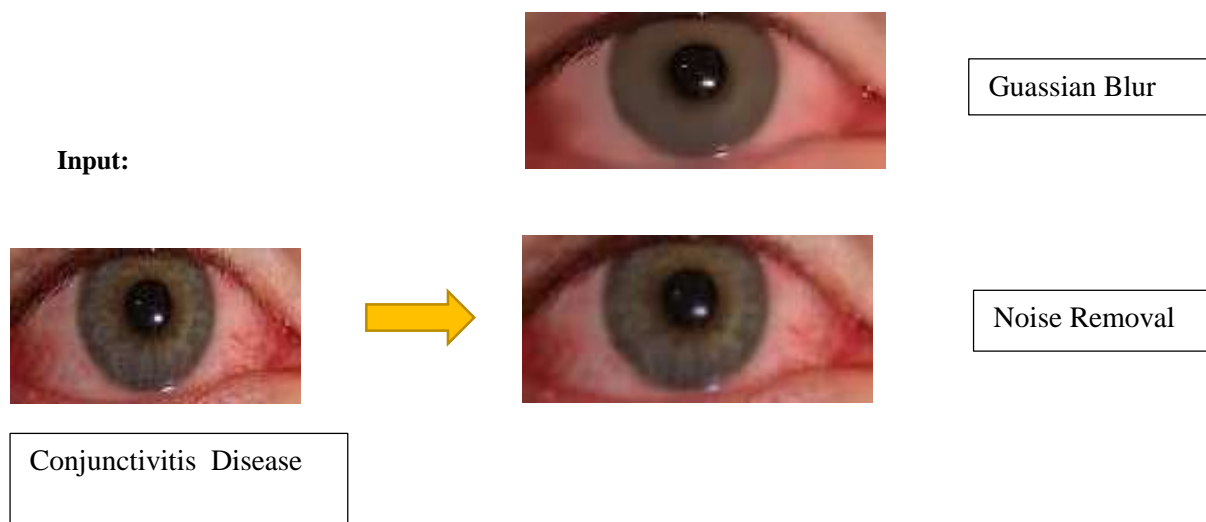




Image Sharpening



Edge Detection

```

print(df.columns.tolist())
Final_result = df.sort_values(0, ascending=False)
print(Final_result)

[0]
4556 255
4064 255
1622 255
4067 255
5060 254
... ..
8967 17
7020 15
156 13
8961 12
7509 5

[29829 rows x 1 columns]
  
```

Pink color Detected

Fig. 2: Image processing and disease detection.

High Throughput Screening (HTS)

Protein responsible for conjunctivitis having Accession id ACFO4411-VP1 is retrieved from NCBI. The homologous templates are selected using BLAST and downloaded from RCSB PDB database.

Using modeller the 3d structure of the protein is modelled.

Modeller generated models were verified using Ramachandran Plot (Table 1, Fig. 2).

Table 1: Ramachandran plot analysis of models

	Core	allowed	generous	outside	
Model 1	280 (91%)	22 (7%)	3 (0%)	0 (0%)	selected



Model 3	273 (89%)	27 (8%)	4 (1%)	1 (0%)	
Model 4	274 (89%)	25 (8%)	4 (1%)	2 (0%)	
Model 5	275 (90%)	29 (9%)	0 (0%)	1 (0%)	

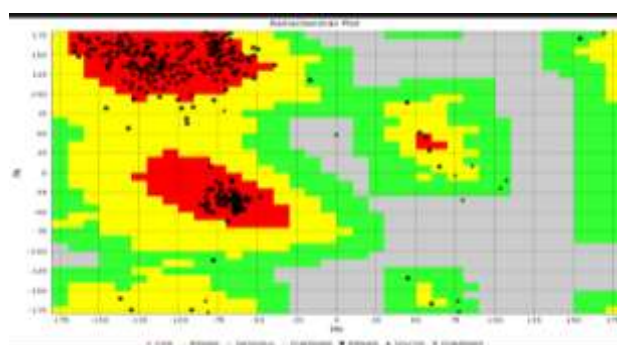


Fig. 2: Ramachandran Plot of Model 1

As per Ramachandran Plot Statistics, we note the maximum number of residues in the core region and least number of residues in the outer region. Hence, VP1.B99990001.pdb is selected and considered for docking studies.

The phytochemicals' SMILES of the medicinal plants eyebright (*Euphrasia officinalis*) and chamomile (*Matricaria chamomilla*) are retrieved from PubChem and ADME screening is performed. Results are noted in Table 2.

Table 2(a): ADME studies of the phytochemicals

Mol Inspiration	miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	volume	nviolations
caffeic acid	0.94	77.75	13	180.16	4	3	2	154.50	0
coumaric acid	-0.36	136.68	23	326.30	8	5	5	278.60	0
chlorogenic acid	-0.45	164.74	25	354.31	9	6	5	296.27	1
hydroxytyrosol	0.52	60.68	11	154.16	3	3	2	141.7	0
Apigenin	2.46	90.89	20	270.24	5	3	1	224.05	0
rutin	-1.06	269.43	43	610.52	16	10	6		3



<i>β-sitosterol</i>	8.62	20.23	30	414.72	1	1	6	456.52	1
---------------------	------	-------	----	--------	---	---	---	--------	---

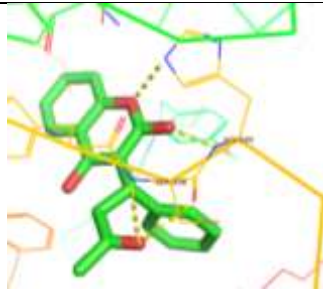
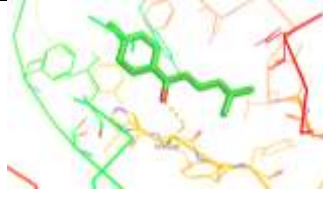
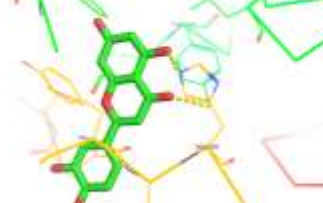
Table 2(b): ADME studies of the phytocompounds

	miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	volume	nviolations
quercetin	1.68S	131.35	22	302.24	7	5	1	240.08	0
luteolin	1.97	111.12	21	286.24	6	4	1	232.07	0
bisabolol	4.68	20.23	16	222.37	1	1	4	248.23	0
chamazulene	4.84	0.00	14	184.28	0	0	1	194.52	0
coumarins	3.03	67.51	23	308.33	4	1	4	277.19	0


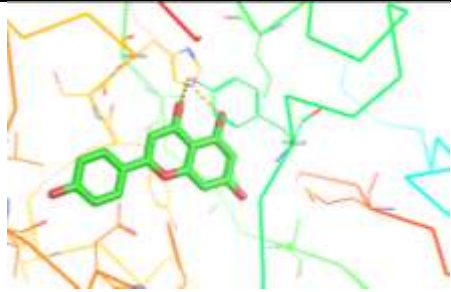
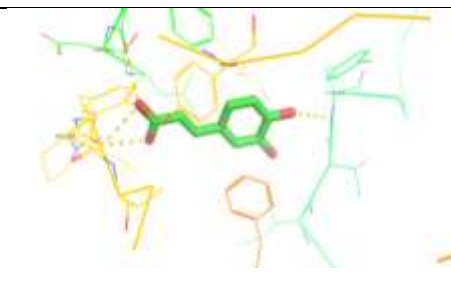
The phytocompounds having nviolations 0 in the table 2 are docked with the receptor protein

VP1.B99990001.pdb generated by modeller. The docking results are given in table 3.

Table 3: Docking

	Docking score	Docking image	Interacting amino acids	No. of interactions
Coumarin	-175.77kcal/mol (MODEL 1)		SER-208 HIS-209 TYR-114	2 1 1
chamazulene	No docking			
bisabolol	-127.97 (MODEL 2)		SER-208	1
Luteolin	-171.71 (MODEL 1)		TYR-114 HIS-209	1 1



quercetin	-182.52 (MODEL 1)		HIS-209 TYR-207	1 2
Apigenin	-163.53 (MODEL 1)		HIS-209 TYR-114	1 1
Caffeic acid	-129.85 (MODEL 1)		TYR-114 PRO-199 TYR-207 ASP-133 PHE-132	1 1 1 1 1

DISCUSSION:

Image pre-processing technique is done improve the quality of raw image. This was done by removing unusual parts from the image background. Further, using image processing technique, we transform the image into digital form and implement certain actions on it, in order to create an improved image. The techniques used are Noise Removal, Guassian Blur, Image Sharpening, Edge Detection of the eye disease image. Further we detect the pinkness in the eye to ascertain that our image is having conjunctivitis or pink eye. Conjunctivitis is detected by pink eye. This was done using Tkinter library and PyCharm online compiler which is tool for python developer.

Next, the gene receptor is retrieved from Genbank and modelled using modeller. As per Ramachandran Plot analysis, Model 1 has most residues in core and least or no residues in outside. With the model,

docking is done. The phytochemicals' SMILES are retrieved from PubChem and ADME screening is done. It is seen that phytochemicals caffeic acid, coumaric acid, hydroxytyrosol, apigenin, quercetin, luteolin, bisabolol, chamazulene and coumarins shows no violations from Lipinski's rule of five and selected for docking studies.

CONCLUSION

As per the docking result it is seen that phytochemical quercetin has least docking score of -182.52Kcal/mol and three interactions with the receptor protein VP1, partial [Coxsackievirus A24] also it is seen that caffeic acid has most five interaction and docking score of -129.85 with the receptor protein. Hence the phytochemicals quercetin and caffeic acid are consider as novel drug lead for conjunctivitis (pink eyes).

FUTURE SCOPE:

© IJPMN, Volume 11, Issue 1, April-2024

(This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution License citing the original author and source)



Further in-vitro receptor ligand binding assay can be done prove to its efficacy as ligand for the disease conjunctivitis.

REFERENCES

1. Hashmi MF, Gurnani B, Benson S. Conjunctivitis. [Updated 2022 Dec 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK541034/?report=classic>
2. Solano D, Fu L, Czyn CN. Viral Conjunctivitis. [Updated 2022 Oct 7]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK470271/>
3. Azari, A. A., & Barney, N. P. (2013). Conjunctivitis: a systematic review of diagnosis and treatment. *JAMA*, 310(16), 1721–1729. <https://doi.org/10.1001/jama.2013.280318>
4. Louten J. (2016). Virus Transmission and Epidemiology. *Essential Human Virology*, 71–92. <https://doi.org/10.1016/B978-0-12-800947-5.00005-3>
5. Haq, A., Wardak, H., & Kraski, N. (2013). Infective Conjunctivitis – Its Pathogenesis, Management and Complications. *Common Eye Infections*. doi: 10.5772/52462
6. Sonka M, Hlavac V, Boyle R, *Image Processing, Analysis and Machine Vision*, 1993
7. Cromey DW. Digital images are data: and should be treated as such. *Methods Mol Biol*. 2013;931:1-27. doi:10.1007/978-1-62703-056-4_1
8. Sarfraz, M. (2020). Introductory Chapter: On Digital Image Processing. In (Ed.), *Digital Imaging*. IntechOpen. <https://doi.org/10.5772/intechopen.92060>
9. Brown BN. (November 2002). "GCI/HRC Research World's First Photograph". *Abbey Newsletter*. Vol. 26, no. 3. Archived from the original on 2019-08-03.
10. "The Birth of Digital Phototelegraphy", the papers of Technical Meeting in History of Electrical Engineering IEEE, Vol. HEE-03, No. 9-12, pp 7-12 (2003)
11. Trussell H & Vrhel M (2008). "Introduction". *Fundamental of Digital Imaging*: 1–6.
12. U.S. Patent 3,277,302, titled "X-Ray Apparatus Having Means for Supplying An Alternating Square Wave Voltage to the X-Ray Tube", granted to Weighart on October 4, 1964, showing its patent application date as May 10, 1963 and at lines 1-6 of its column 4, also, noting James F. McNulty's earlier filed co-pending application for an essential component of invention
13. U.S. Patent 3,289,000, titled "Means for Separately Controlling the Filament Current and Voltage on a X-Ray Tube", granted to McNulty on November 29, 1966 and showing its patent application date as March 5, 1963
14. James RJ (2001). *Scientific charge-coupled devices*. SPIE Press. pp. 3–4. ISBN 978-0-8194-3698-6.
15. Boyle WS; Smith GE. (1970). "Charge Coupled Semiconductor Devices". *Bell Syst. Tech. J.* 49 (4): 587–593. doi:10.1002/j.1538-7305.1970.tb01790.x.
16. Williams, J. B. (2017). *The Electronics Revolution: Inventing the Future*. Springer. pp. 245–8. ISBN 9783319490885.
17. Fossum ER and Hondongwa DB. (2014). "A Review of the Pinned Photodiode for CCD and CMOS Image Sensors". *IEEE Journal of the Electron Devices Society*. 2 (3): 33–43. doi:10.1109/JEDS.2014.2306412.
18. Fossum, Eric R. (12 July 1993). Blouke, Morley M. (ed.). "Active pixel sensors: are CCDs dinosaurs?". *SPIE Proceedings Vol. 1900: Charge-Coupled Devices and Solid State Optical Sensors III*. International Society for Optics and Photonics: 2–14. CiteSeerX 10.1.1.408.6558. doi:10.1117/12.148585. S2CID 10556755.
19. U.S. Patent 4,484,210: Solid-state imaging device having a reduced image lag
20. Fossum, Eric R. (2007). "Active Pixel Sensors". S2CID 18831792.
21. Matsumoto, Kazuya; et al. (1985). "A new MOS phototransistor operating in a non-destructive readout mode". *Japanese Journal of Applied Physics*. 24 (5A): L323. Bibcode:1985JaJAP..24L.323M. doi:10.1143/JJAP.24.L323. S2CID 108450116.
22. Reed, Mike (2002). "Graphic arts, digital imaging and technology education". *T H e Journal*. 21 (5): 69+. Retrieved 28 June 2012.
23. "CMOS Image Sensor Sales Stay on Record-Breaking Pace". *IC Insights*. May 8, 2018. Retrieved 6 October 2019.
24. Murray, Susan (August 2008). "Digital Images, Photo-Sharing, and Our Shifting Notions of Everyday Aesthetics". *Journal of Visual Culture*. 7 (2): 147–163. doi:10.1177/1470412908091935. S2CID 194064049.
25. Castella, T. D. (2012, 1, 12). Five ways the digital camera changed us. *BBC*.
26. Asokan A, Anitha J, Ciobanu M, Gabor A, Naaji A, Hemanth DJ. *Image Processing Techniques for Analysis of Satellite Images for Historical Maps Classification—An Overview*. *Applied Sciences*. 2020; 10(12):4207. <https://doi.org/10.3390/app10124207>
27. Murdikaa U, Darajat AU, and Herlinawati (2022) Performance of edge detection methods for image identification, *AIP Conference Proceedings* 2563, 060002 (2022); <https://doi.org/10.1063/5.0103354>
28. Holzer R, *OpenCV tutorial Documentation*, 2020