Comparative Evaluation of Antimicrobial Activity of Selected Three Herbal Plants Extract with Digital Image Processing Technique

Rajakannu Subashini[1], **Veezhinathan Mahesh**[1], **Anandan Kavitha**[1], **Balasubramaniam Geethanjali**[1], **Sritharan Umamaheshwari Rakshitha**[1]

[1] Department of Biomedical Engineering, SSN College of Engineering

Abstract

Background and Objectives: The development in the technology have witnessed that there is a revival of interest in drug discovery from medicinal plants for treatment of the most destructive diseases. Our investigation characterizes the usage of digital image processing techniques in Matlab to process and analyze the antimicrobial effects of the selected herbal plants.
Methods: The first stage of our investigation involves the extraction of components with methanol from the selected three herbal plants- Solanum xanthocarpum, Solanum nigrum and Helianthus annuus by using soxhlet apparatus. These plant extracts were assayed for antimicrobial activity against 4 different bacterial and fungal species using disk diffusion method.
Results: Notable cell growth inhibitions were observed from the selected microbes. Solanum xanthocarpum exhibits better antibacterial properties on comparison to other two extracts. Solanum nigrum and Helianthus annuus exhibit better antifungal properties by being sensitive factor towards fungal medium. The obtained images were processed using color coding techniques to determine the activity of the extract by isolating the region of inhibition area. The region of inhibition was measured using matlab code and tabulation was compiled to compare the manually measured distances to the automated measurements.
Conclusions: The results provided evidence that the studied plant extracts might indeed be potential sources of natural antimicrobial agents and the introduction of an evaluation technique using image processing was shown to be suitable for the purpose of accurate measurements of zone of inhibition.

Keywords

Solanum xanthocarpum, Solanum nigrum, Helianthus annuus, image processing, antimicrobial activity

Correspondence to:

Mahesh Veezhinathan
Department of Biomedical Engineering
SSN College of Engineering, Kalavakkam
Address: Chennai - 603110
E–mail: maheshv@ssn.edu.in

EJBI 2013; 9(2):14–26
received: January 13, 2013
accepted: March 25, 2013
published: August 30, 2013

1 Introduction

The medicinal plants are the ones whose parts like leaves, seeds, stem, roots, fruits and foliage are used in the preparation of varied extracts, infusions, decoctions and powders in different compositions which are used in the treatment of different diseases of humans, plants and animals and they cause comparatively less side effects and activity of the herbal extracts are considerably high. The use of different parts of several medicinal parts to cure specific ailments has been in vogue from ancient times [1].

Solanum xanthocarpum is a perennial, thorny, herbaceous weeded plant with bright green leaves and zigzag stem, mostly found in arid region. It is commonly known as yellow-berried night shade and also called as Kandankathri in Tamil. It has been widely used in traditional medicine in India and other countries to cure liver disorders, inflammatory conditions, dysmenorrhea, fever, diarrhea, eye diseases, hydrophobia, chronic skin ailments namely psoriasis and ringworm. Crude plant extract is beneficial in bronchial asthma and non-specific cough, influenza, painful and difficult urination, bladder stones and rheumatism [2].

Solanum nigrum commonly known as "Black night shade" belongs to solanacae family. It is called as Manathakkali in Tamil. It shows medicinal properties like an-

timicrobial, anti-oxidant, cytotoxic properties, antiulcerogenic, and hepatoprotective activity. It is an African pediatric plant utilized for several ailments that are responsible for infant mortality especially feverish convulsions, eye diseases, hydrophobia and chronic skin ailments. It is a potential herbal alternative as anti-cancer agent [3].

Helianthus annuus is an important oilseed crop worldwide and commonly called as sunflower. Sunflower seeds are a good source of protein and Vitamins D, E, K, and B. Studies have shown that seeds can prevent invasion of cancer and many more harmful diseases. The Seeds are used as diuretic and expectorant and also used for cough, throat and lung infections. It is a folk remedy for blindness, bronchitis, carbuncles, colic, diarrhea, dysentery, dysuria, eyes, fever, inflammation, laryngitis, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, urogenital ailments and wounds. The flowering head and seeds are febrifuge, nutritive and stomachic. A thick decoction of the roots is used as a warm wash on rheumatic aches and pains [4].

Studies have shown that Solanum xanthocarpum, Solanum nigrum and Helianthus annuus display antimicrobial activity, antioxidant and anti-tumor effect respectively [5, 6, 7]. This reveals pharmaceutically useful phytochemicals enriched in these herbal plants and also suggests that the extraction of active compounds from those plants would be useful to destroy pathogenic microorganisms. The present study has evaluated the antimicrobial activity of methanolic extract of Solanum xanthocarpum, Solanum nigrum and Helianthus annuus on pathological strains. The digital image processing technology and Mat lab tool were used to measure the zone of inhibition and was compared with manually measured distances."

Various image processing routines can be carried out to measure the zone of inhibition and compare with manually measured distances. Digital image processing uses computer algorithms to perform image processing on digital color images. Digital image processing allows a much wider range of algorithms to be applied to the input data and can avoid commonly occurring problems such as the build-up of noise and signal distortion during processing. The output of image processing may be either an image or a set of characteristics or parameters related to the image, which is commonly referred to as extraction of features. Most image-processing techniques involve treating the image as a two-dimensional signal and applying standard signal-processing techniques to it [8].

The application of digital image processing and Mat lab tool for the automation of inhibition zone calculation would give accurate data in the screening of antimicrobial property of herbal plants.

The objective of this study was to evaluate the potential of plant extracts on standard microorganism strains and the obtained results was compared with automated values from digital image processing technique.

2 Materials and Methods

2.1 Collection of Plants and Identification

Seeds of Solanum xanthocarpum, Solanum nigrum and Helianthus annuus were collected from Anna medicinal farm, Chennai. The plants were identified and authenticated by Lifeteck Research Centre, Chennai. The seeds were sorted, cleaned and air dried at room temperature for two weeks and powdered.

2.2 Preparation of plant extracts

2.2.1 Soxhlet Extraction

Solid material containing 50g of the finely powdered plant parts- air dried seeds in the case of Helianthus annuus and dried fruits in case of Solanum xanthocarpum and Solanum nigrum was placed in thick filter paper in thimble. The Soxhlet extractor was placed over a flask containing the extraction solvent. The solvent used in this process was methanol. It is then equipped with a condenser. The solvent was heated to reflux. The solvent vapor travels up a distillation armand floods into the thimble which contains the powdered seeds. The condenser, which occupies the top portion of the setup, ensures that solvent vapor cools, and drips back down into the chamber housing the solid material. The thimble containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm methanol [10]. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the methanol running back down to the distillation flask below. This cycle was repeated for 3 times to get the purified extract. The purified extract was air dried and then weighed to measure the amount of extract obtained from the soxhlet apparatus. The dried extract is then mixed with DMSO, a universal solvent. The quantity of DMSO is approximately equal to one half the total quantity of the extract. This mixture is stored in a air tight storage container and then used for further procedures.

2.3 Antimicrobial Studies

2.3.1 Assay of Antibacterial Activity

The bacterial clinical isolates (Bacillus subtilis, Staphylococcus aureus, Salmonella typhi and Vibrio cholera) were obtained from Lifeteck Research Centre, Chennai.

2.3.2 Preparation of Active Bacterial Suspension

Nutrient broth was made up to 100ml (1.95g in 100ml of distilled water) and was transferred to four different boiling tubes and they were kept for sterilization for

30 minutes in autoclave. This broth was cooled. Active cultures for experiments were prepared by transferring a loopful of stock culture to 25ml of nutrient broth and incubating aerobically at 37°C for 24 hours for bacterial proliferation [11].

2.3.3 Determination of Antibacterial Activity of Herbal Plant Extracts

Muller Hinton agar solid media was used for culturing of bacteria. Agar diffusion assay was carried out to check the antibacterial activity. Muller Hinton agar (6g) was mixed with 300 ml of distilled water along with Agar Agar type1 (3.75g). The medium was sterilized in autoclave at 121°C for 30 minutes and then allowed to cool but not solidify. They were transferred to twelve different sterile petri dishes and the medium was left in the laminar air flow chamber for 30-60 min. After solidification 20μl of the fungal cultures were inoculated to the Petri plates and incubated at 30°C. Freshly prepared sterilized cotton was used to swab the developed bacterial culture onto the solidified medium. Whatman No 3 filter paper discs were impregnated with 20 μl of different concentration of plant extract, kept onto the petri plate and incubated at 37°C for 24 hours. The antifungal activity was assayed by measuring the zone of inhibition for the respective plant extract and it was compared with standard antibiotic. Muller Hinton agar plates without adding cultures were used as control [12].

2.3.4 Assay of Antifungal Activity

The fungal clinical isolates (Rhizopus species, Aspergillus fumigatus, Fusarium and Candida albicans) were obtained from Lifeteck Research Centre, Chennai.

2.3.5 Preparation of Working Fungal Cultures

Potato dextrose broth was prepared by dissolving Potato dextrose agar (3.9g) in 300ml of distilled water and was filtered and the filtrate was transferred to four different boiling tubes and they were sterilized in autoclave at 121°C for 30 minutes. This broth was cooled. Loop full of fungal culture was transferred to the broth and incubating aerobically at 37°C for 24 hours for fungal proliferation [13].

2.3.6 Preparation of Medium

Potato dextrose agar (3.9g) was mixed with 300 ml of distilled water along with Agar Agar type1 (3.75g). It was then sterilized in autoclave for 30 minutes and then allowed to cool but not solidify. Then they were transferred to twelve different sterile petri dishes and the medium was left in the laminar air flow chamber for 30-60 min. After solidification 20 μl of the fungal cultures were inoculated to the Petri plates and incubated at 30°C. Freshly prepared sterilized cotton swabs were used to swab the developed fungal culture onto the solidified medium. Then the filter paper disks immersed in the extract which was serial diluted (sample 1g/100ml of solvent was prepared [10].

Five test tubes with 0.5ml of methanol were taken. 0.5ml of the prepared extract was dissolved in the test tube numbered one and 0.5ml of extract was taken and dissolved in test tube numbered two and the process continues till the test tube numbered five). Hence five different serial dilutions were used. Then they were kept onto the petriplate and observed zone of inhibition for 24-48 hours. The antifungal activity was assayed by measuring the zone of inhibition for the respective plant extract [14]. Potato dextrose agar plates without adding cultures were used as control.

2.4 Digital Image Processing

The images obtained after the antimicrobial effects were processed in Mat lab using digital image processing techniques to differentiate the region of inhibition from the surrounding areas which were prone to bacterial growth. The region of inhibition was around the disc of filter paper immersed in varying concentrations of extracts. This region is represented with dark blue color and the remaining areas with shades of orange and yellow. To measure the region of inhibition from the zoomed picture and to compare the obtained automated values to that of the values that were measured manually.

2.5 Comparative Evaluation of Antimicrobial Images Using Digital Image Processing

In a bitmap, colors were coded on three bytes representing their decomposition on the three primary colors. Colors were interpreted as vectors in a three dimension space where each axis stands for one of the primary colors. The difference between two colors could be quantified by computing the geometric distance between the vectors representing those two colors.

However if there is a very blurry source it should be passed through a sharpness filter first. Each pixel could be compared with its second or third nearest neighbors on the right and on the bottom instead of the nearest neighbors. The edges will be thicker but also more exact depending on the source image's sharpness. There was another way to make edge detection with matrix convolution.

The quality of the results depends on the sharpness of the source image. If the source image was very sharp edged, the result will reach perfection. The other immediate application of pixel comparison was color extraction. Instead of comparing each pixel with its neighbors, we were going to compare it with a given color C1. This algorithm will try to detect all the objects in the image that were colored with C1.

Table 1: Antibacterial activity of *Solanum xanthocarpum*, *Solanum nigrum* and *Helianthus annuus*.

Bacterial species	Zone of Inhibition (cm)		
	Solanum xanthocarpum	*Solanum nigrum*	*Helianthus annuus*
Salmonella typhi	0.11	0.6	1
Bacillus subtilis	0.4	0.5	0.5
Staphylococcus aureus	0.5	0.8	0.7
Vibrio cholera	0.9	0.7	0.6

Table 2: Antifungal activity of *Solanum xanthocarpum*.

Fungal species	Zone of Inhibition (cm)				
	0.5 ml	0.25 ml	0.125 ml	0.0625 ml	0.3125 ml
Fusarium	0.8	0.6	0.5	0.3	0.3
Rhizopus species	0.7	0.6	0.4	0.3	0.3
Candida albicans	0.7	0.5	0.5	0.4	0.3
Aspergillus fumigatus	0.5	0.4	0.4	0.3	0.3

Table 3: Antifungal activity of *Solanum nigrum*.

Fungal species	Zone of Inhibition (cm)				
	0.5 ml	0.25 ml	0.125 ml	0.0625 ml	0.3125 ml
Fusarium	0.4	0.4	0.3	0.3	0.3
Rhizopus species	0.8	0.7	0.6	0.5	0.3
Candida albicans	0.7	0.5	0.4	0.3	0.3
Aspergillus fumigatus	0.8	0.5	0.5	0.4	0.3

Table 4: Antifungal activity of *Helianthus annuus*.

Fungal species	Zone of Inhibition (cm)				
	0.5 ml	0.25 ml	0.125 ml	0.0625 ml	0.3125 ml
Fusarium	0.5	0.4	0.4	0.3	0.3
Rhizopus species	0.5	0.4	0.4	0.4	0.3
Candida albicans	0.6	0.5	0.4	0.3	0.3
Aspergillus fumigatus	1.0	0.9	0.7	0.5	0.4

The Distance calculated using MATLAB displays the Euclidean distance between the two endpoints of the line in a label superimposed over the line and specifies the distance in data units determined by the X Data and Y Data properties, which is pixels. This is used as input for calculating the automated distance which measures the zoomed distance and original distance.

3 Results

Antibacterial and antifungal activity was performed and obtained images were represented in Figure 1, 2, 3, 4, 5, 6. Table 1 represents antibacterial activity of extracts of Solanum xanthocarpum, Solanum nigrum and Helianthus annuus. Zone of inhibition were measured for antibacterial activity. Solanum xanthocarpum showed better activity when compared to Solanum nigrum and Helianthus annuus. Table 2, 3, 4 represents antifungal activity of the extracts. Solanum nigrum and Helianthus annuus exhibit better antifungal activity (the region of inhibition measures a maximum value of 0.5cm in a low concentration of 0.0625ml) when compared to Solanum xanthocarpum. Comparative analysis of manual and automated measurements of region of inhibition was represented in Table 5, 6.

4 Discussion

Solanum xanthocarpum is non-toxic and safe for human use and is regarded as a valuable plant in both Ayurveda and modern drug development areas for its versatile medicinal uses. Solanum nigrum inhibits the growth of several carcinomas. Further studies of other phytoactive compounds will possibly lead to exploration of new

Table 5: Antibacterial assay and Comparative analysis of manual and automated measurements of region of inhibition.

Extract	Name of Species	Zoomed inner diameter (mm)	Zoomed inner radius (mm)	Zoomed outer diameter (mm)	Zoomed outer radius (mm)	Zoom ratio	Obtained distance (manual cm)	Measured Distance (cm)
Solanum nigrum	*Salmonella typhi*	199.13	99.565	1624.94	812.47	180.548	0.5515	0.6
	Bacillus subtilis	172.0500	86.0250	1588	794	172.050	0.4875	0.5
	Staphyllococcus aurus	288.71	144.355	1600	800	177.778	0.8120	0.8
	Vibrio Cholera	204.500	102.250	1605.20	802.600	178.355	0.5733	0.65
Solanum xantho-carpum	*Salmonella typhi*	329.83	164.915	1615.32	807.66	179.48	0.9188	1.05
	Bacillus subtilis	186	93	1830.74	915.37	203.415	0.4572	0.45
	Staphyllococcus aureus	192	96	1614	807	179.33	0.5353	0.5
	Vibrio cholera	236.96	118.48	1670	835	185.556	0.6385	0.7
Helianthus annuus	*Salmonella typhi*	330	165	1702	851	189.111	0.8725	0.95
	Bacillus subtilis	174.050	87.0250	1584	792	176	0.4944	0.5
	Staphyllococcus aureus	270	135	1710	855	190	0.7105	0.7
	Vibrio cholerae	235.42	117.710	1768	884	196.44	0.5994	0.6

methods for therapeutic applications. Helianthus annuus possesses various pharmacological activities. However, it is imperative that more clinical and pharmacological studies should be conducted to investigate the unexploited potential of this plant

The results of the present study showed Solanum xanthocarpum exhibits better antibacterial activity by being sensitive factor towards bacterial medium. The present study reveals that active principles present in plant extracts are active against all the tested bacterial strains. Based on earlier reports, phytochemical compounds found in plants like terpenoids, alkaloids, flavanoids represent the main antibacterial agents [2]. So the antibacterial activity shown by methanolic extracts might be due to some antimicrobial substances present in them [15]. Solanum nigrum and Helianthus annuus exhibit better antifungal activity by being sensitive factor towards fungal medium and the region of inhibition measures a maximum value of 0.5cm in a low concentration of 0.0625ml of both the extracts. They possess better minimum inhibitory concentration values. Antimicrobial properties of extracts against tested microbial strains suggest that the respective crude extracts can be effectively used for common infectious diseases.

Digital image processing techniques were employed and better results were obtained. The color filling technique following the color coding yields a better image which can be distinguished by its individual color for different regions. It makes the region of inhibition more prominent by its individual color from the surrounding microbe prone area and helps in accurate measurement.

5 Conclusion

In conclusion methanol extracts of dried fruits of Solanum xanthocarpum, Solanum nigrum and dried seeds of Helianthus annuus posses a broad spectrum of activity against a panel of bacterial and fungal strains responsible for common infections. Digital image processing techniques were applied on antimicrobial images and various features extracted were analyzed and studied. The comparative analysis measurements of region of inhibition shows that the automated results were nearly equal to the manual counted ones. These promissory extracts open the possibility of finding new clinically effective antimicrobial compounds.

References

[1] Alavijeh PK, Sharma D. A study of antimicrobial activity of few medicinal herbs, Asian Journal of Plant Science and Research. 2012. 2 (4):496-502

[2] Udayakumar R, Velmurugan K, Srinivasan D, Krishna RR. Phytochemical and antimicrobial studies of extracts of solanum xanthocarpum. Anc Sci Life, 2003. 23(2): 90–94.

[3] Singh K, Aali NS, Khan MI, Ahirwar V. Effect of Solanum nigrum on protein content of liver and kidney of albino rats. Pharmacie Globale (IJCP), 2011, 4 (08): 1-3.

[4] Iqbal A, Sadaqat HA, Khan AS, Amjad M. Identification of sunflower (Helianthus annuus, Asteraceae) hybrids using simple-sequence repeat markers. Genetics and Molecular Research. 2010, 10 (1): 102-106.

[5] Kannabiran K, Mohankumar, Gunesekar V. Evaluation of antimicrobial activity of saponin isolated from Solanum xanthocarpum and Centella asiatica. Int J Nat Engg Sci, 2009, 3: 22-25.

[6] Saravanan S, Dhasarathan, P. Gastroprotective and Antioxidant Activity of Solanum nigrum Linn against Aspirin and Cold Restraint Stress induced Ulcerated Rats, Research Journal of Immunology. 2011, 4: 1-11.

[7] Anjali CH, Dash M, Chandrasekaran N, Mukherjee A. Antibacterial activity of sunflower oil microemulsion. International Journal of Pharmacy and Pharmaceutical Sciences. 2010, 2: 123-128.

[8] Lili NA, Khalid F, Borhan NM. Classification of Herbs Plant Diseases via Hierarchical Dynamic Artificial Neural Network after Image Removal using Kernel Regression Framework. International Journal on Computer Science and Engineering, 2011, 3: 15-20.

[9] Pornpanomchai C, Rimdusit S, Tanasap P, Chaiyod C. Thai Herb Leaf Image Recognition System (THLIRS). Kasetsart Journal of Natural Science. 2011, 45: 551-562.

[10] Laurel A, MokaleKognou. Antibacterial and Antioxidant Properties of the Methanolic Extract of the Stem Bark of Pteleopsishylodendron (Combretaceae), Chemotherapy Research and Practice, 2011, 1- 7.

[11] Sidambaram R, Dinesh MG. Antibacterial, antifungal and cytotoxic studies on leaf and seed extracts of Solanum xanthocarpum shrad and wendl, International Journal of Phytopharmacology, 2011, 2(2):61-65.

[12] Sanni S, Onyeyili PA. Phytochemical Analysis, Elemental determination and Some in vitro Antibacterial Activity of Ocimumbasilicum L. Leaf Extracts, Research Journal of Phytochemistry, 2008, 2:77-83.

[13] Sharma D, Lavania AA. In vitro Comparative Screening of Antibacterial and Antifungal Activities of Some Common Plants and Weeds Extracts, Asian J. Exp. Sci, 2009, 23, 169-172.

[14] Cowan, M.M. Plant products as antimicrobial agents, Clinical Microbiology Reviews, 1999, 12 (4): 564–582.

[15] Siddiqui S, Verma S, Rather AA, Jabeen F, Mukesh K. Preliminary phytochemical analysis of some important medicinal and aromatic plants. Advan. Biol.Res, 2009, 3 (5-6): 188-195.

Table 6: Antifungal assay and Comparative analysis of manual and automated measurements of region of inhibition.

Extract	Name of Species	Zoomed inner diameter (mm)	Zoomed inner radius (mm)	Zoomed outer diameter (mm)	Zoomed outer radius (mm)	Zoom ratio	Obtained distance (manual cm)	Measured Distance (cm)
Solanum nigrum	*Fusarium*	140.04	70.02	1670.20	835.10	185.55	0.3772	0.4
		136	68	1674	837	186	0.3656	0.4
		123	61.50	1660	830	184.44	0.334	0.3
		126	63	1666	833	185.11	0.340	0.3
		126	63	1666	833	185.11	0.340	0.3
	Rhizopus species	288.71	144.3550	1600	800	177.77	0.8120	0.8
		270	135	1710	855	190	0.7005	0.7
		199.13	99.565	1624.94	812.47	180.54	0.5515	0.6
		172.07	86.03	1586	793	172.03	0.4885	0.5
		123	61.50	1666	833	184.44	0.333	0.3
	Candida albicans	236.96	118.48	1670	835	185.55	0.6585	0.7
		186	93	1830.74	915.37	203.42	0.4672	0.5
		138	69	1672.20	836.10	186.02	0.3782	0.4
		126	63	1668	834	185.16	0.330	0.3
		124	62	1662	831	184.44	0.334	0.3
	Aspergillus fumigatus	270	135	1710	855	190	0.7105	0.7
		172.05	86.02	1588	794	173.05	0.4775	0.5
		186	93	1830.74	915.370	203.42	0.458	0.5
		123	61.50	1668	834	184.48	0.323	0.3
		124	62	1664	832	184.45	0.334	0.3
Solanum xantho-carpum	*Fusarium*	288.71	144.355	1600	800	177.78	0.8120	0.8
		199.13	99.565	1624.94	812.47	180.54	0.5616	0.6
		172.05	86.025	1588	794	172.05	0.4875	0.5
		123	61.50	1660	830	184.44	0.334	0.3
		130	65	1674	837	187.23	0.322	0.3
	Rhizopus species	270	135	1710	855	190	0.7105	0.7
		204.500	102.250	1605.20	802.600	178.36	0.5733	0.6
		144	72	1670	835	186.55	0.387	0.4
		126	63	1666	833	185.11	0.330	0.3
		124	62	1674	837	187.23	0.332	0.3
	Candida albicans	236.96	118.48	1670	835	185.56	0.638	0.7
		174.050	87.0250	1584	792	176	0.4944	0.5
		186	93	1830.74	915.37	203.42	0.477	0.5
		136	68	1674	837	186	0.376	0.4
		123	61.50	1660	830	184.44	0.334	0.3
	Aspergillus fumigatus	172.050	86.0250	1588	794	172.05	0.4875	0.5
		140	70	1670	835	185.55	0.3772	0.4
		136	68	1674	837	186	0.3666	0.4
		130	65	1674	837	187.23	0.323	0.3
		126	63	1666	833	185.11	0.330	0.3
Helianthus annuus	*Fusarium*	140	70	1670	835	185.55	0.3777	0.4
		144	72	1670	835	186.55	0.387	0.4
		126	63	1666	833	185.11	0.330	0.3
		123	61.50	1666	833	184.42	0.324	0.3
		124	62	1674	837	187.23	0.332	0.3
	Rhizopus species	288.71	144.3550	1600	800	177.78	0.8120	0.8
		270	135	1710	855	190	0.7105	0.7
		199.13	99.565	1624.94	812.467	180.55	0.5715	0.6
		172.05	86.025	1588	794	172.05	0.4875	0.5
		130	65	1674	837	187.23	0.323	0.3
	Candida albicans	236.96	118.48	1670	835	185.56	0.6385	0.7
		192	96	1614	807	179.33	0.5153	0.5
		140	70	1670	835	185.55	0.3872	0.4
		126	63	1666	833	185.11	0.330	0.3
		124	62	1674	837	187.23	0.331	0.3
	Aspergillus fumigatus	288.71	144.3550	1600	800	177.78	0.8120	0.8
		0.4875	172.050	86.025	1588	794	0.4876	0.5
		186	93	1830.74	915.37	203.42	0.4572	0.5
		123	61.50	1666	833	184.44	0.324	0.3
		130	65	1674	837	187.23	0.323	0.3

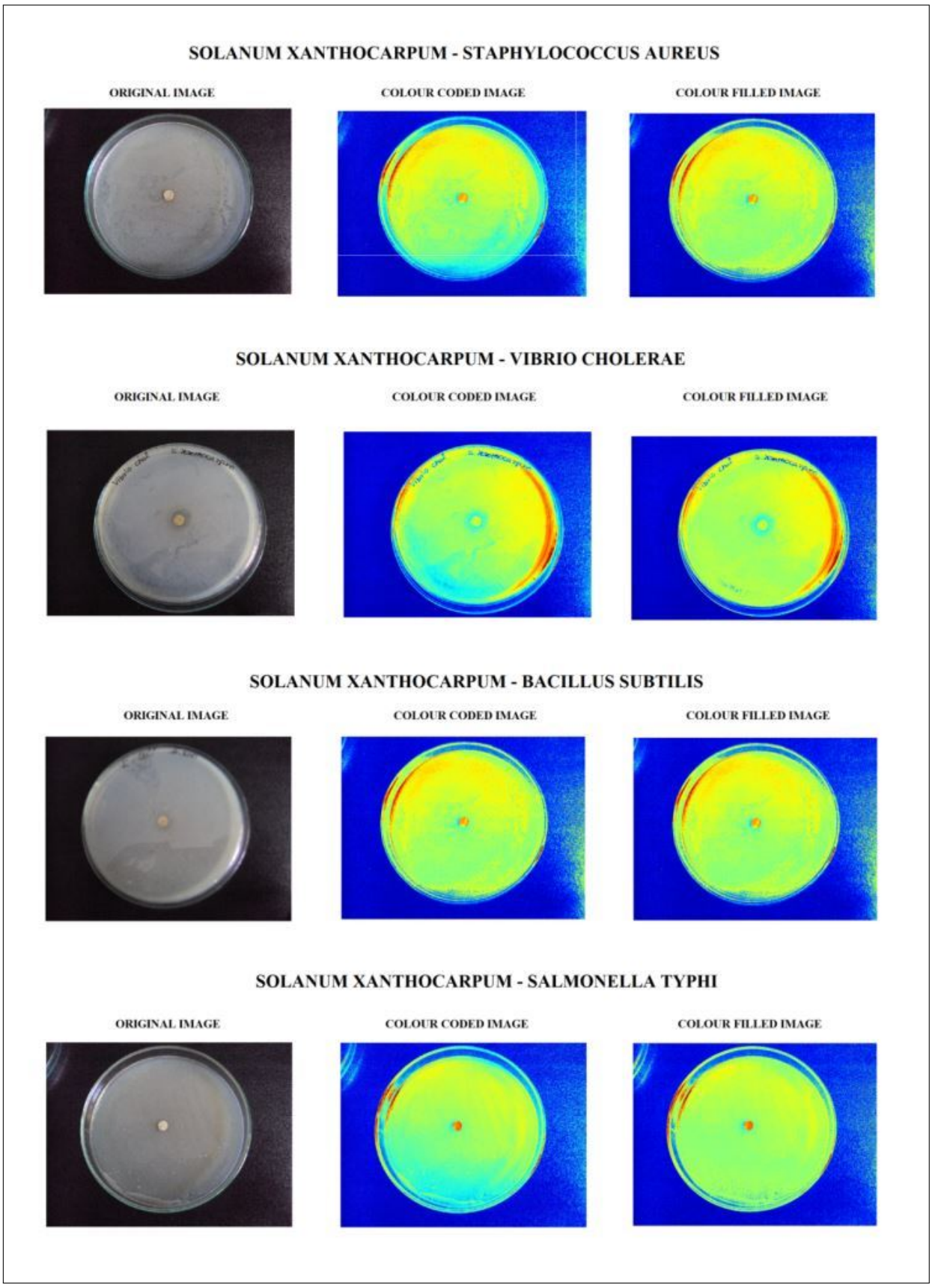

Figure 1: Antibacterial Images of *Solanum xanthocarpum*.

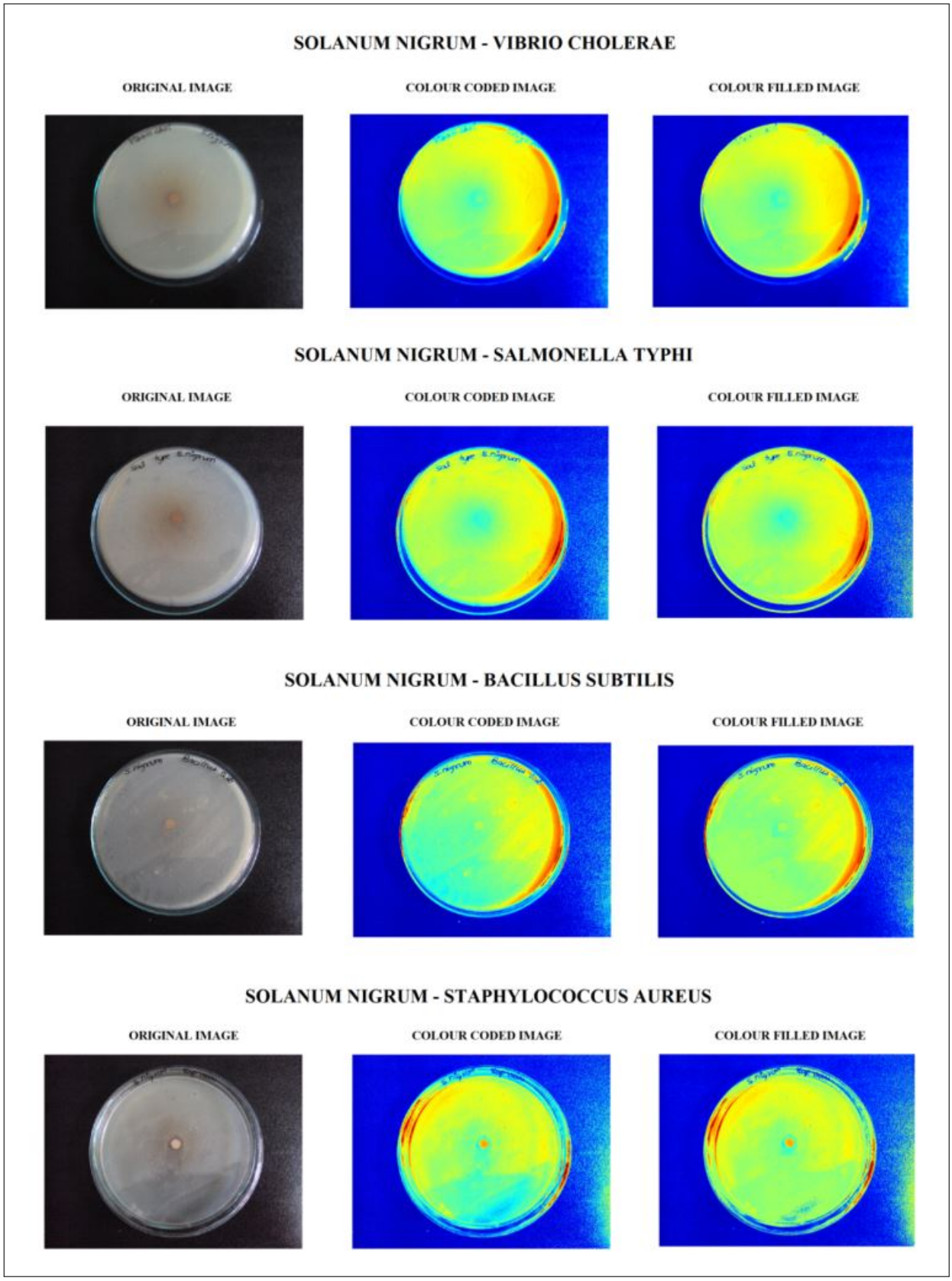

Figure 2: Antibacterial Images of *Solanum nigrum*.

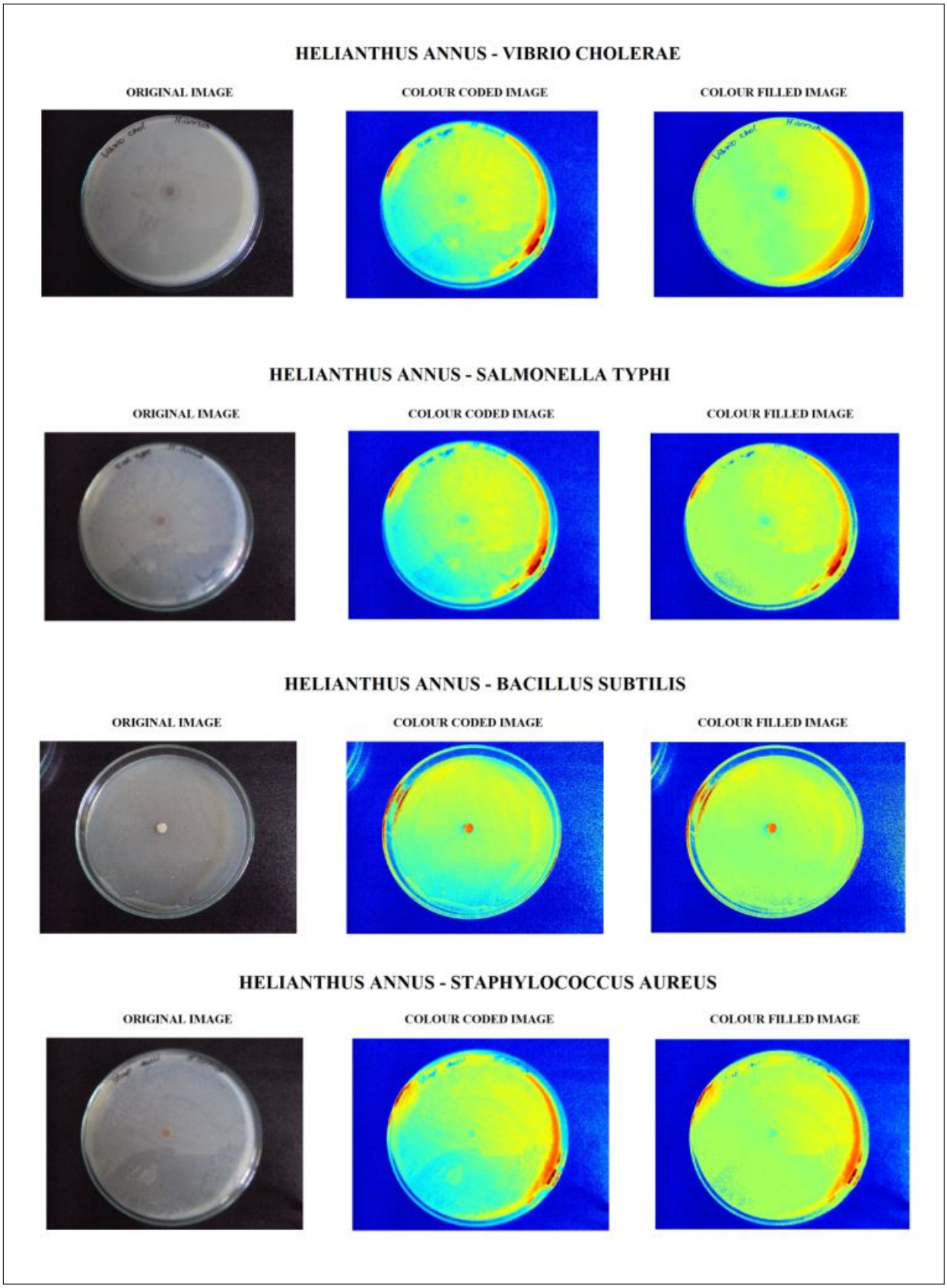

Figure 3: Antibacterial Images of *Helianthus annuus*.

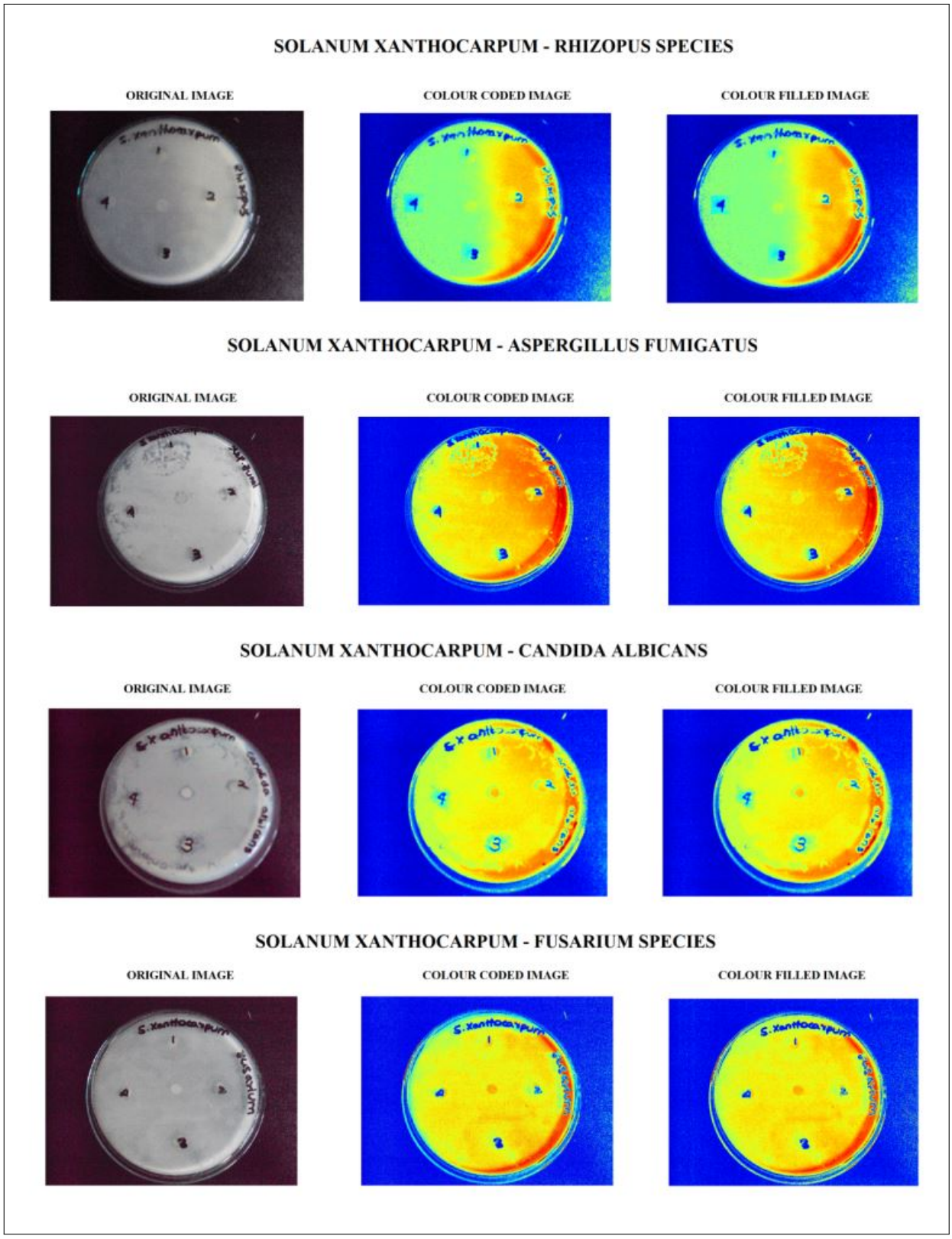

Figure 4: Antifungal Images of *Solanum xanthocarpum*.

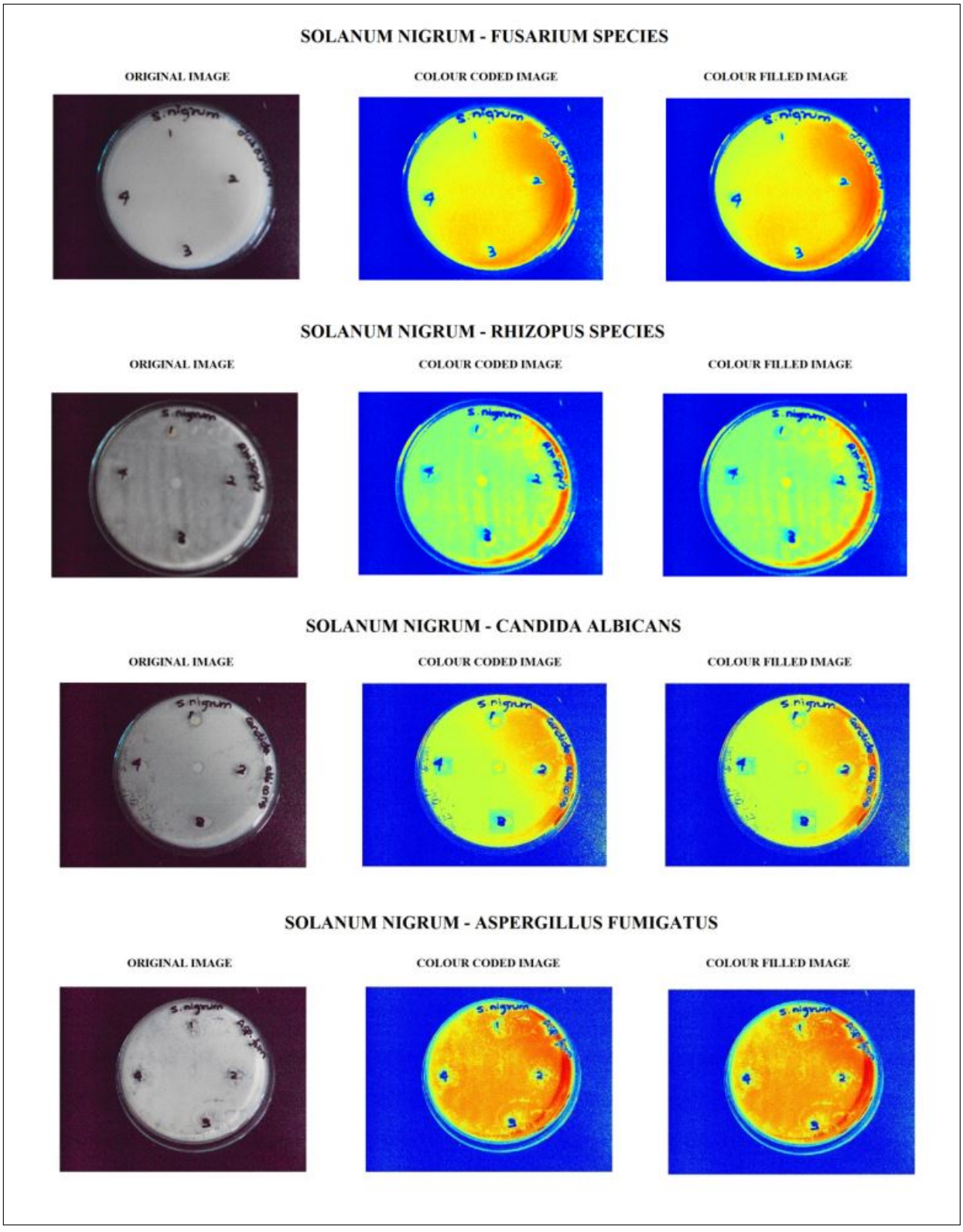

Figure 5: Antifungal Images of *Solanum nigurm*.

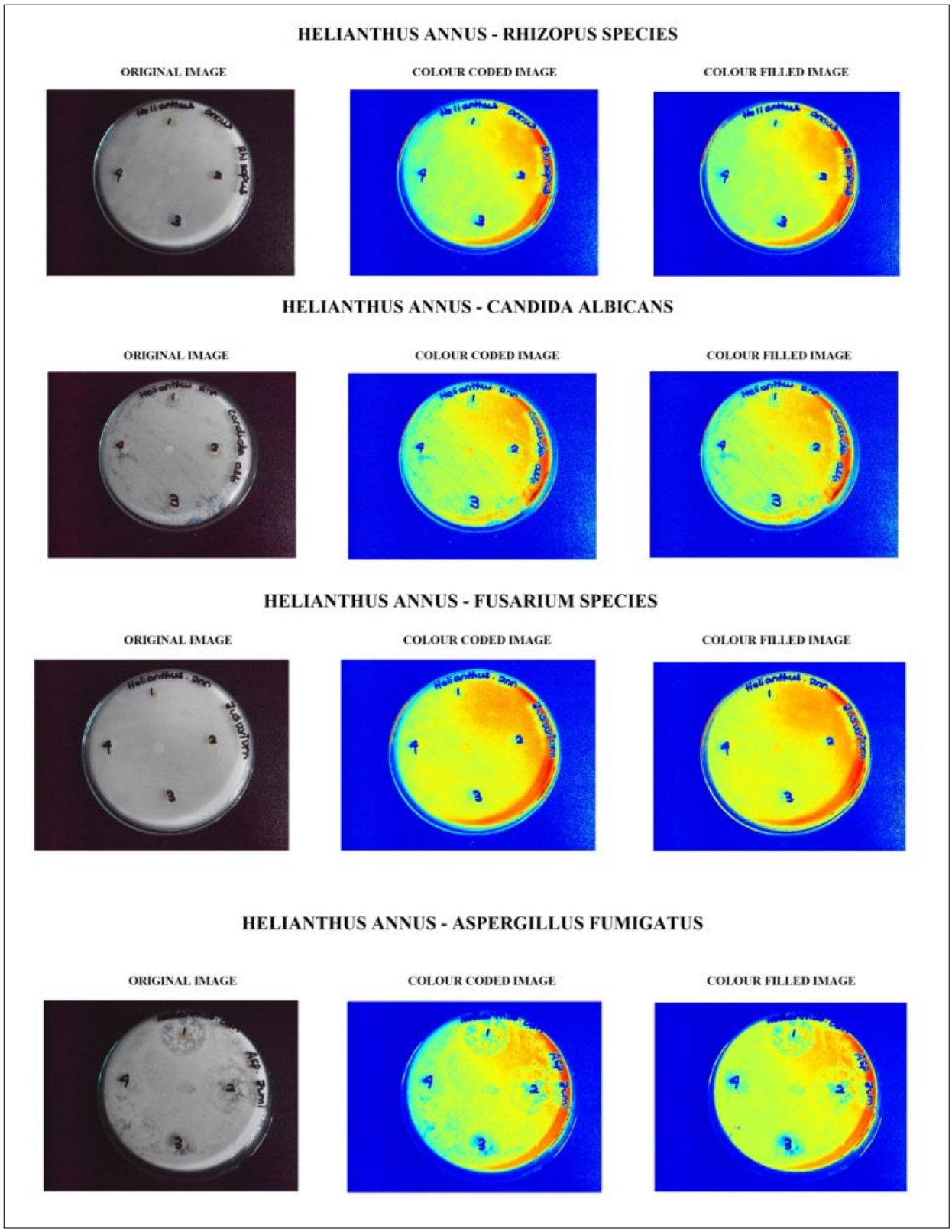

Figure 6: Antifungal Images of *Helianthus annuus*.

