WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 15, 877-890. Research Article ISSN 2277– 7105

COMPARATIVE STUDY OF ANTIFUNGAL ACTIVITY ON HYDRO-ALCOHOLIC LEAVES EXTRACT OF *Psidium guajava* AND *Thespesia populnea* USING PATHOGENIC ORGANISMS

R. Rajeswari*, B. Sasidharan[1], G. Thangaraj[2] and G. Yuvaranjani[3]

[*]Department of Pharmacognosy,

[1-3]Department of Pharamcology

Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Chengalpattu District.

Article Received on
21 July 2023,

Revised on 11 August 2023,
Accepted on 31 August 2023

DOI: 10.20959/wjpr202315-29459

***Corresponding Author**
R. Rajeswari
M.Pharm., Assistant Professor, Department of Pharmacognosy, Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Chengalpattu District.

ABSTRACT

In Humans, fungal infections are one of the most increasingly common diseases in both systemic as well as topical. The present study was performed to compare the antifungal activity of *Psidium guajava* and *Thespesia populnea* using pathogenic organisms and to determine its synergistic effect by combining these extracts. Amphotericin B and Clotrimazole were used as standard drugs to compare the systemic and topical fungal infections respectively. The Hydro-alcoholic extracts of *Psidium guajava* and *Thespesia populnea* were obtained by maceration method using water: ethanol (70:30). The antifungal activity of crude extracts was tested by Agar disc diffusion method and the zone of inhibition was measured. The extract of *Psidium guajava* shows the efficient antifungal activity as compared to the *Thespesia populnea* and there was no synergistic activity was seen.

KEYWORDS: *Psidium guajava*, *Thespesia populnea*, Pathogenic organisms, Antifungal activity.

INTRODUCTION

Medicinal plants also called as medicinal herbs, have been discovered and used in traditional practices since in prehistoric times. In the current situation the efficiency of conventional medicines decreases as the resistance of micro organism increases, it becomes the need to develop new antifungal agents. Present time medicinal plants being the effective source of

medicine. The most common reasons for using herbal plants are that it is more affordable, more closely corresponds to the patient's ideology, and allays concerns about the adverse effects of chemical medicine. The major use of herbal medicine is for health promotion and therapy for chronic diseases.

Psidium guajava [Guava] belongs to the family Myrtaceae and is commonly known as koyyapalam or perakka or jamapandu. It is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various infectious diseases. It has been useful in treating ulcers, sore throat, bleeding gums, malaria and the number of other conditions. These plants contains the enormous amount of phytoconstituents like alkaloids, phenols, tannins, flavonoids, triterpenes, saponins, steroids, carotenoids, vitamins, fiber and fatty acids.

Thespesia poulnea [Indian tulip tree] belongs to the family Malvaceae and is commonly known as Portia tree or Indian tulip tree. It leaves are simple and heart-shaped. The plant is traditionally claimed to possess useful medicinal properties such as antifertility, anti-inflammatory, antioxidant, purgative and hepatoprotective activities. These plants contains the enormous amount of phytoconstituents like tannins, alkaloids, flavonoids, phytosterols, saponins, amino acids etc.

The purpose of the present study was to compare the antifungal activity of *Psidium guajava* and *Thespesia populnea* and to determine its synergistic effect.

MATERIALS AND METHODS

Plant collection and Authentication

The fresh leaves of the plants *Psidium guajava* and *Thespesia populnea* were collected from, in and around near Sothupakkam, Melmaruvathur, Chengalpattu district, Tamilnadu, India. These plants was authenticated by **Dr.K.DHANDAYUTHAPANI, Ph.D., PGDBI**Assistant professor(SG), PG and Research Dept. Of Botany, Aringar Anna Govt. Arts College, Cheyyar. The Reg. no of the Certificate: **AAGAC/BOT/007**.

The fresh leaves were collected in the month of November-2022. The collected leaves were further cleaned to remove the dust and the adhering particles, dried under the shade. The dried material was coarsely powdered by means of mechanical grinder and passed through sieve No.40 and the powdered material was used for further study.

Preparation of extract

Extraction refers to the processes for the isolation of active ingredients from drug material. This may be physical means or by dissolving in a suitable menstruum (liquid solvent e.g., water or alcohol). Extraction is the preliminary step involved in the phytochemical studies. It brings out the primary and secondary metabolites into the extracting solvent depending upon its polarity.

Maceration method

Maceration is the process of softening the tissue and breaking them into pieces using liquids. During maceration, the tissue gets soften and the compound present inside the tissue gets leached out into the liquid(extract). Maceration process involves the separation of medicinally active portions of the crude drugs. It is based on the immersion of the crude drugs in a bulk solvent or menstruum.

The dried coarsely powdered plant leaves of *Psidium guajava* and *Thespesia populnea* were separately extracted by taken these leaves in a glass stoppered flask each weighed 20g and the hydroalcoholic solvent such as water and ethanol in the ratio of 70:30 was pouredinto the three fourth of the flaskand kept in a warm place for 7 days with frequent shaking. The mixture of the crude drug containing solvent was decanted until most of the liquid drains off. Then the resultant filtrate was stand for one day and it was filtered to remove the fine solid particles which are settled down in a flask. The resultant filtrate was evaporated in an electric water bath to obtain the solid residue of extracts. The resulted extracts were tested for further studies.

Phytochemical studies

Phytochemical studies were used to determine the nature of phytoconstituents present in the plant by using suitable chemical tests. It is essenial to study for its pharmacological activities. It can be done by qualitative analysis using specific reagents followed by confirmation with different quantitative estimation and chromatographic techniques like TLC.

A. Chemical Test

The qualitative analysis was very essential to identify the phytochemical constituents in the medicinal plants by using a chemical test such as alkaloids, flavonoids, tannins, triterpenoids, carbohydrates, saponins, steroids and amino acids.

B. TLC

Thin layer chromatography of extracts was done by using standard procedure and is mainly used for the detection of the nature of phytoconstituents presents. In this technique, the different components are separated by the differential migration of solute between two phases.

ANTIFUNGAL ACTIVITY

Principle

The disc diffusion test also known as the Agar disc diffusion test, Kirby-Bauer test, disc-diffusion antibiotic susceptibility test, disc- diffusion antibiotic sensitivity test and KB test is a culture-based microbiology assay used in diagnostic and drug discovery laboratories. It is used to determine the susceptibility of microorganisms.

A pure fungal culture is suspended in saline, its turbidity is standardized, and it is swabbed uniformly across on agar plate. An antibiotic- or extract-impregnated filter paper disk is then placed on the surface of agar. The disc constituent(s) diffuse from the filter paper into the agar. The concentration of these constituents will be highest next to the disk and will decrease as the distance from the disk decreases. If the antibiotic or extract is affective against certain fungi at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the **zone of inhibition.** In general, larger zones of inhibition correlate with lower minimum inhibitory concentrations (MICs) of antibiotic or extract for that fungal strain.

Media

The disc diffusion method is performed using Sabouraud Dextrose Agar (SDA) is used for the cultivation of fungi (yeasts, molds). This medium is also employed to determine microbial contamination in food, cosmetics, and clinical specimens. The pH is adjusted to approximately 5.6 in order to enhance the growth of fungi, especially dermatophytes.

Sabouraud Dextrose Agar contains digests of animal tissues (peptones) which provide a nutritious source of amino acids and nitrogenous compounds for the growth of fungi and yeasts. Dextrose is added as the energy and carbon source. Agar is the solidifying agent. High dextrose concentration and low pH favor fungal growth.

Composition of Sabouraud Dextrose Agar (SDA)

Dextrose- 40.0 g/L

Peptone- 10.0 g/L

Agar- 15.0 g/L

Final pH (at 25°C) 5.6±0.2

Preparation of Sabouraud Dextrose Agar (SDA)

1. Suspend 65g of the medium in one liter of distilled water or deionized water.
2. Heat with frequent agitation and boil to completely dissolve the medium.
3. Sterilize by autoclaving at 121° C for 15 minutes.
4. Check the pH of each preparation after it is sterilized, which should be between 7.2 and 7.4 at room temperature.
5. Cool the agar medium to 45 to 50°C.
6. Pour the agar into sterile glass petri dish on a flat surface to a uniform depth of 4 mm.
7. Allow to solidify.
8. Prior to use, dry plates at 30-37°C in an incubator, with lids partly a jar, for not more than 30 minutes or until excess surface moisture has evaporated. Media must be moist but free of water droplets on the surface. Presence of water droplets may result to swarming microbial growth, which could give inaccurate results. They are also easily contaminated.

Preparation of inoculum

Stock culture were maintained at 4°C sabouraud dextrose agar slant. Active cultures for experiments were prepared by transferring the stock cultures into the test tubes containing sabouraud dextrose broth, that were incubated at 48 hrs at room temperature. The assay was performed by agar disc diffusion method.

Microorganisms chosen under the study

Fungi

1. *Aspergillus niger*
2. *Candida albicans*
3. *Mucor mycosis*
4. *Trichophyton rubrum*

Standard drugs chosen under the study

1. Amphotericin-B

2. Clotrimazole

Amphotericin-B was chosen as the standard drug under our study for the systemic fungal infections while as the Clotrimazole for the topical fungal infections in order to compare the effectiveness of the extracts in both systemic as well as topical.

Procedure

Sabouraud Dextrose agar (SDA) medium is poured into the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Samples were placed over the plate. Amphotericin-B is taken as positive control. The plates were incubated at 37°C for 24 hours. Then antifungal activity was determined by measuring the diameter of zone of inhibition.

RESULTS

Since the herbal medicines are prepared from the plant origin in the form of infusion, decoction or extraction, in such cases they are prone to contamination which leads to deterioration of phytoconstituents and variation in chemical composition. Hence, before proceeding to the pharmacological screening or clinical trials, the scientists or research scholars need to authenticate the phytoconstituents of herbal plants to detect their potency. The number of analytical techniques were developed for quality control of crude drugs.

Here the phytochemical investigation plays a major role in understand the dynamics of herbal plants and also to develop the quality parameters. In this study different phytoconstituents were extracted from the dried coarsely powdered leaves of *Psidium guajava* and *Thespesia populnea* by using solvents water and ethanol (70:30) by maceration method.

The percentage yield of hydro-alcoholic extracts of leaves of ***Psidium guajava***was found to be **21.64%w/w** and ***Thespesia populnea***was found to be **16.37%w/w.**

The hydro-alcoholic extracts of dried powdered leaves of ***Psidium guajava***was showed that the extract was **dark brown** in colour with **semi-sticky** in nature and ***Thespesia populnea*** showed that **chocolate brown** in colour with **semi-solid** in nature.

S.No	Name of the drug	% yield	Consistency	Colour
1.	***Psidium guajava***	21.64%w/w	Semi-sticky	Dark brown
2.	***Thespesia populnea***	16.37%w/w	Semi-solid	Chocolate brown

Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the presence of phytoconstituents in each extract.

The hydro-alcoholic extracts of leaves of ***Psidium guajava*** and ***Thespesia populnea*** showed the maximum number of primary and secondary metabolites as follows, **Carbohydrates, Flavonoids, Tannins, Triterpenoids, Amino acids, Alkaloids, Triterpenoids and Steroids.**

S.No	Chemical test	*Psidium guajava* dried leaf powder	*Thespesia populnea* dried leaf powder	*Psidium guajava* leaves extract	*Thespesia populnea* leaves extract
1.	Carbohydrate	+	+	+	+
2.	Alkaloids	+	+	+	+
3.	Tannins	+	+	+	+
4.	Triterpenoids & Steroids	+	+	+	+
5.	Flavonoids	+	+	+	+
6.	Amino acids	_	+	+	+
7.	Saponins	+	+	+	+

Qualitative chromatographic analysis for these extracts of plants by thin layer chromatography method was performed to separate and identify the single or mixture of phytoconstituents present in each extract.

The hydro-alcoholic extract of leaves of ***Psidium guajava*** was found to be the **R_f Value** for **tannins, flavonoids, triterpenoids and phenolic compounds** was found to be **0.22, 0.733,0.812 and 0.858** respectively.

The hydro-alcoholic extract of leaves of ***Thespesia populnea***was found to be the **R_f Value** for **tannins, flavonoids, triterpenoids and phenolic compounds** was found to be **0.06, 0.470,0.76 and 0.47** respectively.

S.No	Active constituents	Solvents	*Psidium guajava*	*Thespesia populnea*
1.	Flavonoids	Ethyl acetae:Formic acid:Water (24:3:3)	0.73	0.76
2.	Tannins	Chloroform:Ethyl acetate:Methanol (5:3:2)	0.22	0.06
3.	Triterpenoids	Chloroform:Methanol (20:10)	0.81	0.56
4.	Phenol	Chloroform:Ethyl aceate (30:3)	0.85	0.47

IN-VITRO*STUDY OF ANTI-FUNGAL ACTIVITY OF HYDRO-ALCOHOLIC LEAVES EXTRACTS OF *Psidium guajava* AND *Thespesia populnea.

Table no.: Antifungal activity of Hydroalcoholic extract of *Psidium guajava* for different pathogens.

	zone of inhibition in mm					
S.No	**Organism**	**500µg/ml**	**750 µg/ml**	**1000 µg/ml**	**Amphotericin B**	**Clorimazole**
1	*Aspergillus niger*	6mm	7mm	8mm	10mm	8mm
2	*Candila albicans*	4mm	6mm	8mm	9mm	8mm
3	*Mucor mycosis*	5 mm	6 mm	7 mm	9 mm	6mm
4	*Trichophyton rubrum*	4 mm	7 mm	8 mm	8 mm	6mm

Table no.: Antifungal activity of Hydroalcoholic extract of *Thespesia populnea* for differentpathogens.

Zone of inhibition in mm						
S.No	**Organism**	**500µg/ml**	**750µg/ml**	**1000µg/ml**	**Amphotericin B**	**Clotrimazole**
1	*Aspergillus niger*	6 mm	8 mm	9 mm	8 mm	8 mm
2	*Candida albicans*	-	-	-	9 mm	7 mm
3	*Mucor mycosis*	-	-	-	7 mm	7 mm
4	*Trichophyton rubrum*	-	-	-	8 mm	6 mm

Table no.: Antifungal activity of Hydroalcoholic extracts of *Psidium guajava* and *Thespesia populnea* for different pathogens.

Zone of inhibition in mm						
S.No	**Organism**	**500µg/ml**	**750µg/ml**	**1000µg/ml**	**Amphotericin B**	**Clotrimazole**
1.	*Aspergillus niger*	6 mm	7 mm	8 mm	10 mm	9 mm
2.	*Candida albicans*	-	-	-	10 mm	7 mm
3.	*Mucor mycosis*	5 mm	6 mm	7 mm	9 mm	7 mm
4.	*Trichophyton rubrum*	5 mm	7 mm	8 mm	8 mm	6 mm

DISCUSSION

The project entitled **"Comparative Study of Antifungal Activity on Hydro-Alcoholic Leaves Extract of *Psidium guajava* and *Thespesia populnea Using Pathogenic Organisms*"** has been achieved by the following results.

The literature review has been showed the adequate information in relating to the ***Psidium guajava***and ***Thespesia populnea*** extracts. It revealed that the leaves of these plants showed the maximum amount of Flavonoid, Tannins, Triterpenoid, Alkaloids and Phenolic content. It was reported that the plant flavonoids and tannins may contribute to several anti-fungal

mechanisms. Flavonoids, anti-fungal activity is probably due to their ability to complex irreversibly with nucleophilic aminoacids in proteins, often leading to inactivation of the protein and loss of function. This information on individual plant extracts gave a clue to carry out this study.

The qualitative phytochemical studies in the extracts of the plants showed the maximum number of phytoconstituents. Their presence was further confirmed by TLC profiling of each plant extract, which shows different spots representing various constituents of flavonoids, tannins, triterpenoids and phenolic acids.

The extracts exhibit a significant antifungal activity against pathogenic fungi including the resistant microorganisms, the zone of inhibition increases with increase in concentration.

CONCLUSION

This research work shows that *Psidium guajava*, *Thespesia populnea* has the potential bioactive phytochemicals that are responsible for the anifungal activity. It also proven that *Psidium guajava* has more potent than *Thespesia populnea.* From the above study it was concluded that the extracts shows the significant antifungal acivity against pathogenic micro organisms including resistant micro organisms, the zone of inhibition increases with increase in concentration. The individual extracts shows the significant antifungal activity but there was no synergistic activity was seen. In future further investigation are required on isolation of bioactive compounds from the above extracts to study the pharmacological action. The above information creates an interest for the research scholars to carry out the work in future.

Compliance with ethical standards

ACKNOWLEDGMENTS

The authors wish to thanks the Sasam Biological Lab Services, Chennai, for their assistance during the labwork.

REFERENCES

1. Alfred goodman gilman, Theodore W.Rall, Alan S. Nies, Palmer taylor. The Pharmacological Basis of Therapeutics. 8thed. New York:Maxwell Macimillan International Editions.p.1166-79.

2. Amit pandey, Shweta. Antifungal Properties of Psidium guajava Leaves and fruits against Various Pathogens. Journal of Pharmaceutical and Biomedical Sciences 2011; 13(16): 2230-85.
3. Anju Dhiman, Arun Nanda, Sayeed Ahmad, Narasimhan B. *In vitro* antimicrobial activity of methanolic leaf extract of *Psidium gujava* L. Journal of Pharmacy and BioAllied Sciences 2011; 3(2): 226-229.
4. Atanukumardas, Nazrul islam, Omar faruk, Ashaduzzaman, Rudi Dungani. Review on tannins: Extraction Processes, applications and possibilities. South African Journal of Botany, 2020: 58-70.
5. Balsano C, Alisi A. Antioxidant effects of natural bioactive compounds. Curr Pharm. 2009 Dec; 15: 3063-73.
6. Batubara, Suparto H, Wulandari N.S. The Best Extraction Technique for Kaempferol and Quercetin Isolation from Guava Leaves (Psidium guajava). IOP Publishing, 2017; 1315-55.
7. Beckett A.H, Stenlake J.B. Practical Pharmaceutical Chemistry. CBS publishers, 4th ed, 2001; 115-126.
8. Brua D. Clayton, Yuvonne N. Stock. Basic Pharmacology for Nurses. 10th ed, Missouri: Mosby Year- Book, Inc., 1989; 478-88.
9. Chandran R.P, Manju S, Shaji P.K, Nair G.A and Sukumar B. In Vitro Cytotoxic Activities of Leaf Extracts of Thespesia populnea and Hygrophillaschulli Against Dalton's Lymphoma ascites and Ehrlich Ascites Carcinoma Cell Lines. Journal of Lung Cancer Research, 2016; 1(2): 1007.
10. Chas patel V, Kulkarni J.S, Chaudhari A.B, Chincholkar S.B. Foundations in Microbiology. 7th ed, Pune: NiraliPrakashan, 2011; 6.10-6.15.
11. Chetan S pandanaboina. Alcohol Stress on Cardiac Tissue- ameliorative effects of Thespesia populnea leaf extract, Journal of Cardiology, Nov, 2013; 63(6).
12. Chih-Cheng Lai, Che-Kim Tan, Yu-Tsung Huang, Pei-Lan Shao, Po-Ren Hsueh. Current challenges in the management of invasive fungal infections. Journal of Infection and Chemotherapy, 2008; 14(2): 77-85.
13. Chumbhale D.S. Phytochemical, Pharmacological and Phytopharmaceutics Aspects of *Thespesia populnea* (linn.) soland.: A Review. Inventi Rapid: Ehnopharmacology, 2010; 1(2).

14. Dolores vargasalvarez, Marcos sotohernandez, Victor A gonzalezhernanddez, Mark engleman E, Agustin damian Nava. Flavonoids in Psidium guajava L. Horticulture International Journal., 2021; 5(1): 38-41.
15. Don A. Ballington, Mary M. Laughlin. Pharmacology for Technicians. 2nded, Chennai: New Age International (P) Limited, 2005; 375-377.
16. Donald C. Sheppard, Scott G. Filler. Host Cell Invasion by Medically Important Fungi. Cold Spring Harb Perspect Med., 2015; 5(1): a019687.
17. Eva sanchezarmengol, Meisaharmanci, Flavia laffleur. Current strategies to determine antifungal and antimicrobial activity of natural compounds. Microbial Research, 2021 Nov.
18. Gokhale S.B, Kokate C.K, Bidarkar D.S. Pharmaceutical Biology (Remedial Biology), 6th ed. Pune: NiraliPrakashan, 2010; 101-3.
19. Goodman and gilman's. The Pharmacological basis of therapeutics. 10thed.New York: Medical Publishing Division, 2008; 1295-310.
20. Harborne J.B. Phytochemical mehods. 3rd ed, 1998; 291-293.
21. Ilango K, Valentina P. Medicinal Chemistry – III. 1sted.Chennai: Keerthi publishers, 2020; 144-59.
22. Ilavarasan R. Analgesic and Anti-Inflammatory Properties of Thespesia populnea. Natural Product Research, 2012; 26(17): 1616-20.
23. Imtiyaz Wani. Pharmaceutical Microbiology. 1sted.New Delhi:S.Vikas and Company (Medical publishers), 2018; 138-43.
24. Jain N.K. Pharmaceutical Microbiology. 2nded.Delhi: M.K Jain for Vallabh Prakashan, 2005; 62-7.
25. Kokate C.K, Purohit A.P, Gokhale S.B. Pharmacognosy. Nirali prakashan, 54th ed, Pune, 2017; Appendices.A.22-27.
26. Kruti N pandya, Alok J Shah, Sonal S Patel, Vidhi B Parikh, Ghanshyam R Patel, Hardik K Soni. Evaluation of Safety and In-Vitro efficacy Study of Anti-Fungal Cream. Journal of Advanced Pharmacy Education and Research, Jul, 2013; 3(3).
27. Kusum kaushik, Shweta agarwal. Role of Herbal Antifungal agents For the Management of Fungal Diseases: A Systematic Review. Asian Journal of Pharmaceutical and Clinical Research, Jul, 2019; 12(7).
28. Manika Das, SubhagataGoswamia. Antifungal and Antibacterial Property of Guava (Psidium guajava) Leaf Extract: Role of Phytochemicals. International Journal of Health Science and Research, 2019; 9(2): 2249-71.

29. Manoj kumar, Maharishi Tomar, Ryszard Amarowicz, Vivek Saurabh, M. Sneha Nair, Chirag Maheswari, Minnu Sasi, Uma Prajapati, Muzaffar Hasan, Surinder Singh, Sushil Changan, Rakesh Kumar Prajapat, Mukesh K. Berwal, Varsha Satankar. Guava (*Psidium guajava* L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioacivities. Foods, 2021; 10(4): 752.
30. Meigynelcemailoa, Meta mahendradatta, Amaranlaga, NatsirDjide. Tannin Extract of Guava Leaves (Psidium guajava L) Varaiation with Concentaration Organic Solvents. International Journal of Scientific and Technology Research, 2013; 2(9): 2277-86.
31. Mohammed Saleh Al Aboody, Suresh Mickymaray. Ani-Fungal Efficacy and Mechanisms of Flavonoids. Antibiotics (Basel), 2020; 9(2): 45.
32. Muthukumar S and Sami Veerappa N. Phytochemical analysis in the root and leaf of Thespesia populnea (Linn) Soland ex correa. Journal of Pharmacognosy and Phytochemistry, 2018; 7(1): 414-7.
33. Oluwajobiiyanuloluwa, Kabiruyusufadamu, Jigam A Audu. Antibacterial And Antifungal activities of aqueous leaves extract of some medicinal plants. GSC Biological and Pharmaceutical Sciences, 2019; 09(01): 62-9.
34. Padron- Marquez beatriz, Viveros- Valdez ezequiel, Oranday- Cardenas azucena, Carranza- Rosales pilar. Antifungal Activitiy of Psidium guajava Organic Extracts against dermatophytic fungi. Journal of Medicinal Plants Research, 2012; 6(41): 5435- 38.
35. Parthasarathy R, Amrendersingh, Debjitbhowmik. In Vitro Antioxidant Activity Of bark and Leaf of Thespesia Populnea. Research Journal of Pharmacognosy and Phytochemistry, 2016; 8(1).
36. Pratap Chandran R, manju S, Vysakhi M.V, Shaji P.K, Achutam Nair G. Antibacterial and Antifungal Activities of Thespesia populnea Leaf Extracts against human pathogens. International journal of Pharmtech Research, 2014; 6(1): 290-297.
37. Prawestydiahutami, Herinsetianingsih, Indira firdhasyafitri, Rico pratamawiyono. The Anti- Malarial Effect of Thespesia populnea (L.) Soland ex Correa Extract Using Malaria Mice Model Infected with P.berghei. Pharmacognosy Journal, 2021; 13(2): 585-590.
38. Rajamurugan. R. GC-MS analysis, antibacterial and antibacterial activities of Thespesia populnea Linn. Leaf in-vivo study. BiomedRx., 2013; 1(3): 248-53.
39. Rang H.P, Dak M.M, Ritter J.M, Moore P.K. Pharmacology, 5th ed. New Delhi:Elsevier, A division of Reed Elsevier India Private Limited, 2003; 666-70.
40. Rang H.P, Dale M.M, Ritter J.M, Flower R.J. Pharmacology, 6th ed. Churchill Livingstone Elsevier, 2007; 692-7.

41. Rataboli P.V. Clinical Pharmacology and Rational Therapeutics, 2nd ed. New Delhi:Ane Book Pvt. Ltd., 2010; 361-4.
42. Richard A. Harvey, Pamela C. Champe. Pharmacology, 4th ed. New Delhi: Wolters Kluwer (India) Pvt. Ltd., 2006; 407-10.
43. Salil K. Bhattacharya, Parantapasen, Arunabharay. Pharmacology, 2nd ed. New Delhi: Elsevier (A Division of reed Elsevier India Pvt. Ltd.), 2004; 447-51.
44. Sampath kumar, Norizamhdsarbon, Sandeep singhrana, Anjani devichintagunta, Prathibha S, Satheesh kumaringilala, Jeevan kumar S.P, Sai anvesh, Vijaya ramudirisala. Extraction of Bioactive compounds from Psidium guajava leaves and its utilization in preparation of Jellies. AMB Express, 2021; 11(36).
45. Sangeetha R. A New Serine Protease from the Leaves of Thespesia populnea. Preparative biochemistry and Biotechnology, 2013; 43(1): 95-107.
46. Sangeetha R, Vedasree N. *In Vitro* α-Amylase Inhibitory Activity of the Leaves of *Thespesia populnea*. ISRN Pharmacology, 2012; 515634.
47. Sarita Kesarkar, Amol bhandage, Smitadeshmukh, Kavita shevkar, Mukta abhyankar. Flavanoids: An Overview. Journal of Pharmacy Research, 2009; 2(6): 1148-54.
48. Shanmugam krishnamoorthy, Gnanaprakasamadaikalaraj, Manivachagamchandrasekaran. Antibacterial and Antifungal Activity of Leaves of Thespesia populnea. International Journal of Pharmaceutical Science, 2014; 6(8): 404-11.
49. Shivakumarhugar. Wound healing activity of the leaves of Thespesia populnea. Journal of Natural Remedies, 2007; 7(1): 120-124.
50. Sumra naseer, Shabbir hussain, Naureennaeem, Muhammad pervaiz and Madiharahman. The Phytochemistry and Medicinal Value of Psidium guajava (guava). Springer Open., 2018; 4(32).
51. Tripathi K.D. Essential of Medical Pharmacology. 5thed.New Delhi: Jaypee Brothers Medical publishers (p) Ltd, 2004; 715-24.
52. Vasudevan M, Parle M. Pharmacological actions of Thespesia populnea relevant to Alzheimer's disease. International Journal of Phytotherapy and Phytopharmacology, 2006; 13: 9-10.
53. Vinay kumar, Abuk K Abbas, Nelson fausto. Pathogenic Basis of Disease. 7th ed. New Delhi: Elsevier India Pvt. Ltd., 2004; 397-410.
54. Vinod D Rangari. Pharmacognosy and Phytochemistry, 1st ed, 2008; 129-139.
55. URL:https://byjus.com
56. URL:https://en.m.wikipedia.org/wiki/Antifungal

57. URL:https://en.m.wikipedia.org/wiki/Aspergillus-niger
58. URL:https://en.m.wikipedia.org/wiki/Fungal_infection
59. URL:https://en.m.wikipedia.org/wiki/Fungus
60. URL:https://en.m.wikipedia.org/wiki/Medicinal-plants
61. URL:https://en.m.wikipedia.org/wiki/Mucormycosis
62. URL:https://en.m.wikipedia.org/wiki/Psidium_guajava
63. URL:https://en.m.wikipedia.org/wiki/Trichophyton-rubrum
64. URL:https://keys.lucidcentral.org/keys/v3/eafrinet/weeds/key/weeds/Media/Html/Psidium-guajava-(Guava).htm
65. URL:https://microbenotes.com/sabouraud-dextrose-agar-sda/
66. URL:https://my.clevelandclinic.org/health/diseases/22961-candida-albicans
67. URL:https://uses.plantnet-project.org>Thespesiapopulnea
68. URL:https://www.efloraofgandhinagar.in/tree/thespesia-populnea
69. URL:https://www.healthline.com/health/fungal-infection
70. URL:https://www.investindia.gov.in/team-india-blogs/phytopharmaceuticals-india-opportunity
71. URL:https://www.mdpi.com/2304-8158/10/4/752
72. URL:https://www.medicalnewstoday.com/articles/322722
73. URL:https://www.stylecraze.com/articles/benefits-of-guava-leaves-for-skin-hair-and-health/