VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 379-386 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Evaluation of *In Vitro* Anti-tubercular and Anti-inflammatory Potentials of Fruit Coat Extracts of *Michelia champaca*

Sivakumar D.

Department of Pharmaceutical Chemistry, PSG College of Pharmacy, Coimbatore, Tamil Nadu, India

Received: 1st February, 2024; **Accepted:** 22nd May, 2024; **Published online:** 17th August, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.036

Abstract: *Michelia champaca* is an evergreen or semi-deciduous plant native to South Asia. It is predominantly found in India and also found in countries like Indonesia, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam. Literature Survey provides little scientific evidence in its pharmacognostical and pharmacological properties. The present study aimed to evaluate the phytochemical and the biological activities of various extracts prepared from fruit coats of *Michelia champaca.* The fresh fruit coats were collected, dried and extracted using a Soxlet extractor. The *in vitro* anti-tubercular activity and anti-inflammatory activity of dried fruit coat extract was performed. Physicochemical constants like moisture content, extractive values, ash values were carried out. Preliminary phytochemical and HPTLC screening of the extracts showed the presence of alkaloids, phenols, flavonoids, tannins, and steroids. All the extracts exhibited concentration-dependent anti-tubercular effect and anti-inflammatory activity. The study was conducted in order to find possible isolated compounds as a bio-source for future novel anti-tubercular formulation.

Keywords: Anti-tubercular study, *Michelia champaca*, Electrophoresis, Phytochemical screening, Alamar Blue

Citation: Sivakumar D.: Evaluation of *in vitro* anti-tubercular and anti-inflammatory potentials of fruit coat extracts of *Michelia champaca.* Intern. J. Zool. Invest. 10(2): 379-386, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.036

Introduction

Tuberculosis is caused by *Mycobacterium tuberculosis*, which is a highly contagious disease transmitted via air. *M. tuberculosis* survives in crowded living conditions and among the malnourished; it is a disease with the highest impact in developing countries. The global burden of Tuberculosis (TB) is quite large, even with the development of efficient antibiotics and other synthetic drugs. Improvement of socioeconomic status in the 20th century helped reduce mortality rates in developed countries, but TB continues to be a major scourge especially in the developing world. The TB incidence and fatality rate are noticed in the age group of 15-49 due to malnutrition (Chikhalia *et al*., 2006). In 2014, an estimated 9.6 million people developed TB which includes 5.4 million men, 3.2 million women and 1.0 million children. Globally about 1.5 million deaths occurred (among them 0.4 million HIV-positive and 1.1 million HIV-negative people). In

2014 an approximated 480 000 women, 890 000 men and 140 000 children died because of TB (Chilamakuru *et al*., 2013).

TB is the most leading disease causing many deaths among the AIDS patients. This becomes further more dangerous due to incurable extensive drug resistant (XDR) TB and multi drug resistant (MDR) TB which causes serious mortality. The MDR and XDR strains are unresponsive to the presently available drugs, which represents a serious medical problem. Despite of many drugs available for the treatment of TB, a compound with novel and potent mechanism of action is still not available (Desai *et al*., 1999).

M. tuberculosis is primarily a pathogen of the mammalian respiratory system, which infects the lungs. *M. tuberculosis* is an acid-fast bacterium. It is highly aerobic and requires high levels of oxygen. It does not produce spores, and is non-motile. *M. tuberculosis* divides every 15–20 h. It has an unusual, waxy coating on its cell surface due to the presence of mycolic acid, the primary reason for its drug resistance. It is a small bacillus that can survive in a dry state for weeks.

Humans are the only known reservoirs of *M. tuberculosis*. The organism survives intra-cellular, have long periods of metabolic inactivity and tend to develop resistance to single drug. Therefore, combined drug therapy is employed to delay the emergence of this resistance (Patel *et al*., 2011). It is thus important to search for new and more potent compound with novel mode of action to reduce the emergence of drug resistance against *Mycobacterium tuberculosis.*

Traditional herbal medicines are safe and efficient for the treatment of diseases and infections. Thus medicinal plants are used worldwide due to its easy availability and efficacy. *Michelia champaca* L. (family Magnoliaceae) is commonly called as Champak or Golden Champa. It is wild in the forests of the eastern sub Himalayan tract and lower hills up to 3000 ft and also in Assam, Myanmar, South India and Western Ghats. Leaves are simple, alternate, petiole 1 to 3 cm long, elliptic, lanceolate, spiral, and reticulate. Flowers are solitary, yellow, dull-yellow when fresh, orange when old and fragrant. In India this plant is cultivated in gardens and near the temples for its fragrant flowers and handsome foliage. Its volatile oil is highly esteemed in perfumery and in useful in cephalalgia, ophalagia, ophthalmia, gout and rheumatism.

Taprial *et al*. (2013) studied antifertility effect of hydroalcoholic leaves extract of *Michelia champaca* L. Jarald *et al.* (2008) studied Anti diabetic activity of flower buds of *Michelia champaca* Linn. Vimala *et al.* (1997) studied anti-inflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract.

The present study aimed to evaluate the phytochemical and the biological activities of various extracts prepared from fruit coats of *Michelia champaca.* The *in vitro* anti-tubercular activity and anti-inflammatory activity of dried fruit coat extract was performed.

Materials and Methods

Collection and preparation of plant material:

The plant material was collected from the Kalapatti village, Coimbatore, Tamil Nadu, India. The collected fruit coats of *Michelia champaca* was wiped with a clean white cloth to remove the extraneous materials adhered to it. The fruit coats were air-dried thoroughly under shade (at room temperature) for three weeks to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and then homogenized to coarse powder. Then it was stored in air-tight containers for further analysis.

Physicochemical Investigations (*Khan et al., 2002; Jarald et al., 2008; Ahmad et al., 2012; Parimi and Kolli, 2012*):

Total Ash:

2 g of the dried fruit coat powder of *Michelia*

champaca was accurately weighed. The ground drug was scattered in a fine even layer at the bottom of the tarred crucible and incinerated, by gradually increasing the temperature to 450°C in a muffle furnace till it is white (indicating the absence of carbon). Then it was cooled and weighed. The percentage of ash was calculated with reference to the amount of air dried crude drug.

Extractive Values:

Macerate five grams of the air-dried coarsely powdered crude drug with 100 ml of solvent of the specified strength in a closed flask for 24 h, shaking frequently during the first 6 h and allowing to stand undisturbed for the next 18 hours. Thereafter, filter rapidly taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105 °C, and weigh. The percentage of the extract with reference to the air dried drug was calculated.

As per the above procedures the extraction was done, by using the following solvents in increasing polarity:

Chloroform < Ethyl acetate < Methanol < Hydro-alcohol < Water

Extractive values was found out by

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant extract}} \times 100$$

Preliminary Phytochemical Analysis:

Preliminary phytochemical analysis was done for all the extracts to investigate the primary and secondary metabolites. The qualitative chemical tests were done for alkaloids, tannins, phenolics, glycosides, flavonoids and sterols, to detect various phytoconstituents present in the fruit coat of *Michelia champaca* by using the standard methods.

In Vitro Screening of Anti Tubercular Potentials:

Microplate Alamar Blue Assay Method:

The anti-mycobacterial activity of compounds were assessed against *M. tuberculosis* (H37Rv strain) using Microplate Alamar Blue Assay (MABA). 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of extracts were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After 5 days, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as bacterial growth. The MIC is defined as lowest drug concentration which prevented the colour change from blue to pink.

The standard drugs used were Pyrazinamide, Ciprofloxacin and Streptomycin, in the concentration range 100 µg/ml to 0.2 µg/ml.

Evaluation of In Vitro Anti-Inflammatory Activity by Gelatin Zymography (*Snoek-van Beurden and Von den Hoff, 2005; Frankowski et al., 2012; Phanse et al., 2012; Vijayalakshmi et al., 2012; Bae et al., 2015; Nishijima et al., 2015; Ghasemian et al., 2016; Shaikh et al., 2016; Ren et al., 2017*):

Many different types of zymography exist according to the type of enzyme. For the specific case of proteases (MMP-2, MMP-9) gelatin is one of the most frequently used substrate. Proteolytic activity was determined by the presence or absence of clear bands over a deep blue background, after Coomassie staining. For the preparation of MMP samples, the tissue sample was chopped completely, added 5 ml of tris buffer, centrifuged at 3000rpm for 15 min, and stored it at -20°C for further use. For the preparation of extract added 50 µl of MMP sample and 50 µl of the extract/compound (the sample given for study) and incubated for one hour. Mixed this and added non-reducing buffer in equal volume. The Negative control was 50 µl of MMP and Positive control was 50 µl of MMP sample +50 µl of Diclofenac sodium (stored for 1 h). Cleaned

electrophoresis apparatus in warm water and cleaned glass plates in Methanol.

Set up plates. Large plate, then two spacers (applied little petroleum jelly on both sides of the Spacers) and small plate on top. Assembled plates into clamp and gently tightened the screws. Heated the agarose gel, poured between the two glass plates just to seal the bottom surface, left it for 5-10 min. Prepared the resolving gel as given above. Mixed the appropriate resolving gel mixture and pipette between the glass plates avoiding bubbles. Filled plates about 80% way up, leaving space for the stacking gel and comb. Overlay with a small amount of water to achieve a completely flat interface between resolving gel and stacking gel. Allowed to set for about 45 min. While resolving gel was setting, prepared the stacking gel as given above. When resolving gel was set, poured off the excess water and added wash between the plates with distilled water. Poured in stacking gel and inserted comb avoiding bubbles. Allowed to set for about 30 min. When stacking gel was set gently removed comb, washed the wells with distilled water and assembled gels onto the electrode/gasket section of the gel apparatus. Filled top and bottom of the tank with reservoir buffer i.e. upper tank with 100 ml and lower tank with 150 ml. Loaded 20 µl of sample in each well. Connected the electrodes. Put lid on tank and plug cables into power supply. Run at about 50V for 15 min and then 100 V until the bromo-phenol blue reached the bottom of the plates. After electrophoresis, dissembled the apparatus and gently removed the gel and put into a plastic dish and wash the gel with zymogram renaturing buffer i.e. 2.5%Triton x-100 for one hour to remove SDS from the gel and allow proteins to denature. Decant the zymogram renaturing buffer and incubated the gel in zymogram incubation buffer at 370 °C overnight. Stained with Coomassie blue R-250 for one hour. Gels should be de-stained with an appropriate Coomassie R-250 de-staining solution for about 2 h. After staining, the background stains blue with Coomassie stain where the gelatin was degraded. White bands appeared indicating the presence of gelatinases. The lower bands are gelatinases-A(MMP-2) which was about 72KD while the upper bands were gelatinases-B(MMP-9) which runs at about 95KD.

Results

Qualitative Phytochemical Screening:

Preliminary phytochemical analysis was done by performing identification tests to the extracts of fruit coat of *Michelia champaca.* Ethyl acetate extract showed the presence of phytosterols, Chloroform extract showed the presence of coumarins, Methanol extract showed the presence of alkaloids, tannins and phenolics, Hydro-alcoholic extract showed the presence of phytosterols, tannins and phenolics, Aqueous extract showed the presence of glycosides (Table 1).

In vitro Anti Tubercular Activity (*Taneja and Tyagi, 2007; Guzman et al., 2010; Sanusi et al., 2017; Sharifi-Rad et al., 2017*):

In-vitro Anti- tubercular activity for all the extracts (S1, S2, S3, S4 and S5) was done by microplate Alamar Blue Assay method, using sterile 96 well plates. *Mycobacterium tuberculosis* (H37Rv strain) was used for this assay. For all the extracts DMSO was used as the vehicle. Middlebrook 7H9 broth was employed as the nutrient medium. The standard drugs used were Pyrazinamide, Ciprofloxacin and Streptomycin, in the concentration range 100 µg/ml to 0.2 µg/ml. All the extracts were tested for activity at concentration range from 100 µg/ml to 0.8 µg/ml and compared with standards of same concentration range. The results of this experiment were noted by presence or absence of growth of $H37_{RV}$ strain of *Mycobacterium tuberculosis* as shown in Table 2. At the end of sixth day the results were observed, after adding Alamar Blue. Alamar blue is a blue colour dye which changes into pink colour in the presence of oxygen.

Blue colour indicates absence of growth of bacteria, i.e. sensitive to the plant extract. Pink colour indicates growth of bacteria, i.e. resistant to

Table 1: Preliminary phytochemical analysis

Sample No.	Test	Ethyl -acetate Extract	Chloroform Extract	Methanol Extract	Hydro -alcohol Extract	Aqueous Extract
A1	Sterols	_	_	_	+	_
A2	Tannins	_	_	+	+	_
A3	Saponins	_	_	_	_	_
A4	Coumarins	_	+	_	_	_
A5	Glycosides	_	_	_	_	+
A6	Phenolics	+	_	+	+	_
A7	Alkaloids	_	_	+	_	_

Table 2: *In vitro* anti tubercular activity of various extracts prepared from fruit coat of *Michelia champaca*

S. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	**S1**	S	S	S	S	S	R	R	R
2	**S2**	S	S	S	S	S	S	S	R
3	**S3**	S	S	S	S	S	S	S	R
4	**S4**	S	S	S	S	S	S	S	R
5	**S5**	S	S	S	R	R	R	R	R

S = Sensitive **R** = Resistant

the plant extract. All the extracts were active against the bacteria at a concentration range of 100 µg/ml to 25 µg/ml, which was indicated by the presence of blue colour as shown in the Figures 1 and 2.

Anti-Inflammatory Activity by Gelatin Zymography:

In vitro anti-inflammatory activity for all the extracts (A1, A2, A3, A4 and A5) was done by Gelatin zymography method. Matrix metallo-proteinase (MMP-2 and MMP-9) enzyme was used for this assay. For all extracts DMSO was used as vehicle. The standard drug used for this study was Diclofenac sodium. Sodium dodecyl sulfate-polyacramide (SDS-PAGE) gel was used for the electrophoresis. All the extracts were run in the gel along with the standard drug, then the zymogram was compared and the results were analyzed as shown in Table 3. The results of this experiment were noted by the presence or absence of the proteolytic activity, which appears as clear bands over a deep blue background, after Coomassie staining. Appearance of white clear band indicates the absence of proteolytic actvity i.e. the extract does not have anti-inflammatory activity as shown in Figure 3.

Absence of band indicates the presence of proteolytic activity i.e. the extract has anti-inflammatory activity. All the extracts had the property of proteolysis, hence significant anti-inflammatory activity. The activity was represented in the form of percentages.

Discussion

Preliminary phytochemical evaluation of different solvent extracts of fruit coat of *Michelia champaca* exhibited the presence of various secondary metabolites. These secondary metabolites have numerous pharmacological properties such as

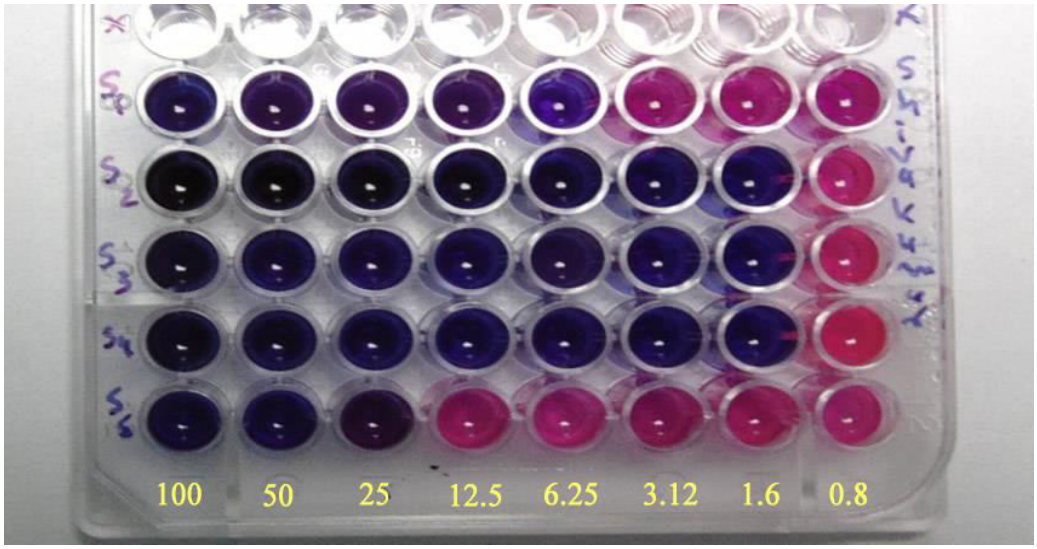

Fig. 1: Wells treated with test extract solutions.

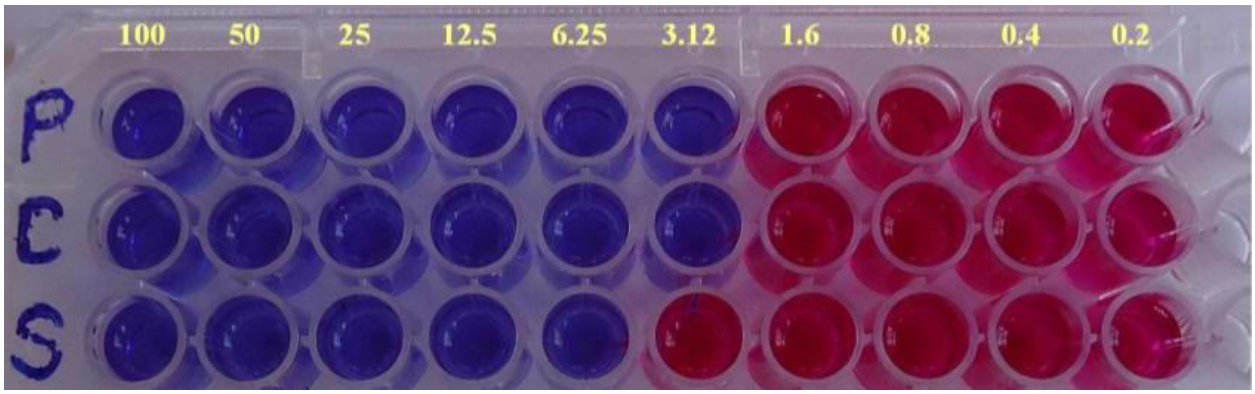

Fig. 2: Wells treated with standard drug solutions (P - Pyrazinamide , C - Ciprofloxacin, S – Streptomycin).

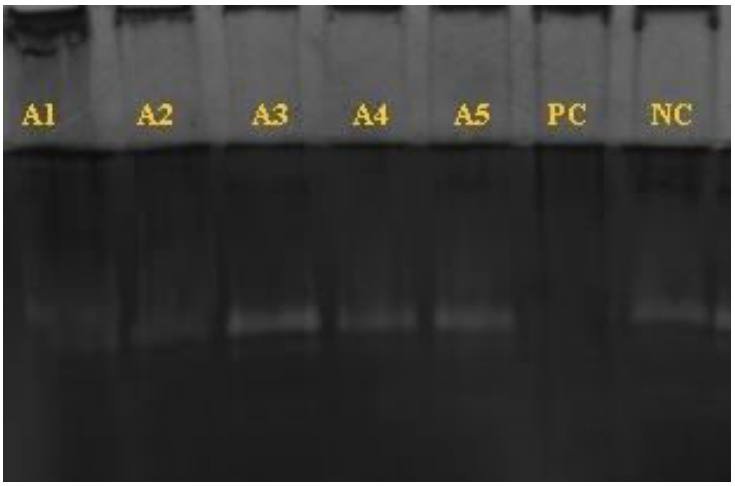

Fig. 3: Anti-inflammatory activity of various extracts prepared from fruit coat of *michelia champaca* by gelatin zymography.

Table 3: *In vitro* anti inflammatory activity of various extracts prepared from fruit coat of *michelia champaca*

S. No.	Sample	Anti-inflammatory activity against MMP-2(%)	Anti-inflammatory activity against MMP-9(%)
1	**A1**	85	75
2	**A2**	90	80
3	**A3**	60	40
4	**A4**	65	55
5	**A5**	68	60
6	**Positive Control**	100	95
7	**Negative Control**	10	NIL

antibacterial, anti-inflammatory, hepatoprotective, anti-allergic, anticancer, anti-diabetic, insecticidal, anti-malarial, and anti-viral properties. So the present work, was done to analyze the preliminary phytoconstituents and to evaluate the biological activities of various extracts prepared from fruit coat of *Michelia champaca* . In preliminary phytochemical analysis the fruit coats of *Michelia champaca* contains Phytosterols, Coumarins, Alkaloids, Phenolics, Tannins and Glycosides**.** The *In vitro* Anti-tubercular activity was evaluated by 96 micro well plates Alamar blue Assay (MABA). All the extracts have significant anti-tubercular activity. The MIC of Ethyl acetate extract, chloroform extract and hydro-alcoholic extract was found to be 1.6 µg/ml. Whereas MIC of the standard drugs were found as Pyrazinamide - 3.125 µg/ml, Ciprofloxacin - 3.125 µg/ml, Streptomycin - 6.25 µg/ml. The reports of MIC clearly gives evidence on the significant property of extracts to inhibit H37Rv strain of *Mycobacterium tuberculosis* and since the MIC is 1.6 µg/ml (lesser concentration than standard), the phytoconstituents present in the fruit coat of *Michelia champaca*, has the greater potential of inhibiting *Mycobacterium tuberculosis* when compared to standard drugs. The *In vitro* anti-inflammatory activity was done by Gelatin Zymography. The percentage activity of inhibition of Matrix Metalloproteinase of all the extracts were evaluated and among them the maximum percentage activity was shown by Aqueous extract with 90% and 80% for MMP-2 and MMP-9, respectively. Whereas the percentage activity of standard drug (Diclofenac sodium) for MMP-2 and MMP-9 was found to be 100% and 95%, respectively. From the above results of percentage activity, it is evident that the phytoconstituents present in the extracts of dried fruit coats of *Michelia champaca* inhibit Matrix Metalloproteinase, one of the enzymes which cause inflammation.

Finally to conclude, it was found that *Michelia champaca* has a variety of phytoconstituents that have potent anti-inflammatory and anti-tubercular properties based on the experiments carried out. This provides scientific support for further research into the plant's lead compounds and efforts to conduct *in vivo* models.

References

Ahmad H, Sehgal S, Mishra A, Gupta R and Saraf SA. (2012) TLC detection of β-sitosterol in *Michelia champaca* L. leaves and stem bark and it's determination by HPTLC. Pharmacogn J. 4(27): 45–55.

Bae MJ, Karadeniz F, Ahn B-N and Kong C-S. (2015) Evaluation of effective MMP inhibitors from eight different brown algae in human fibrosarcoma HT1080 cells. Prev Nutr Food Sci. 20(3): 153-161.

Chikhalia KH, Desai PS, Desai KR and Patel B. (2006)

Synthesis of pyrimidine based thiazolidinones and azetidinones: Antimicrobial and antitubercular agents. Indian J Chem. 45(B): 773-778.

Chilamakuru Naresh B, Shankarananth V, Rajasekhar KK and Triveni S. (2013) Synthesis, characterization and antitubercular activity of some new 3,5 disubstituted 2,4-thiazolidinediones. Asian J Pharmaceut Clin Res. 6(5): 29-53.

Desai RM, Desai JM and Shah V H. (1999) Synthesis and anti-microbial profile of 1,3,4-oxadiazoles, sulphonamides, 5-Imidazolinones, Azometines, 4-Thiazolidinones, 2- Azetidinones, formazanes and tetrazolium chlorides. Indian J Heterocyclic Chem. 4: 329-334.

Frankowski H, Gu YH, Heo JH, Milner R and Del Zoppo GJ. (2012) Use of gel zymography to examine matrix metalloproteinase (gelatinase) expression in brain tissue or in primary glial cultures. Methods Mol Biol. 814: 221-233.

Ghasemian M, Owlia S and Owlia MB. (2016) Review of anti-inflammatory herbal medicines. Adv Pharmacol Sci. 2016: 1-11.

Guzman JD, Gupta A, Evangelopoulos D, Basavannacharya C, Pabon LC, Plazas EA, Muñoz DR, Delgado WA, Cuca LE, Ribon W, Gibbons S and Bhakta S. (2010) Anti-tubercular screening of natural products from Colombian plants: 3-methoxynordomesticine, an inhibitor of MurE ligase of *Mycobacterium tuberculosis*. J Antimicrob Chemother. 65(10): 2101-2107.

Jarald E, Joshi S and Jain D. (2008) Antidiabetic activity of flower buds of *Michelia champaca* Linn. Indian J Pharmacol. 40(6): 256.

Khan MR, Kihara M and Omoloso AD. (2002) Antimicrobial activity of *Michelia champaca*. Fitoterapia ;73(7–8): 744-748.

Nishijima CM, Delella FK, Rodrigues CM, Rinaldo D, Lopes-Ferreira MV, da Rocha LR, Vilegas W, Felisbino SL and Hiruma-Lima CA. (2015) The anti-inflammatory effects of the methanolic extract and fractions from *Davilla elliptica* St. Hil. (Dilleniaceae) on *Bothrops jararaca* Envenomation. Int J Mol Sci. 16(6): 12454-12466.

Parimi U and Kolli D. (2012) Antibacterial and free radical scavenging activity of *Michelia champaca* Linn. flower extracts. Free Radicals Antioxidants 2(2): 58-61.

Patel D, Patel N, Kumari P and Patel N. (2011) Synthesis and characterization of some new azetidin-2-ones containing coumarin moiety and their antimicrobial study. Int J Chem. 3(2): 117-123.

Phanse MA, Patil MJ, Abbulu K, Chaudhari PD and Patel B. (2012) In-vivo and in-vitro screening of medicinal plants for their anti-inflammatory activity: an overview. J Appl Pharm Sci. 2(7): 19-33.

Ren Z, Chen J and Khalil RA. (2017) Zymography as a research tool in the study of matrix metalloproteinase inhibitors. Methods Mol Biol. 1626: 79-102.

Sanusi SB, Abu Bakar MF, Mohamed M, Sabran SF and Mainasara MM. (2017) Southeast asian medicinal plants as a potential source of antituberculosis agent. Evidence-Based Complem Altern Med. 2017: 1–39.

Shaikh RU, Pund MM and Gacche RN. (2016) Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system *in vitro* as well as *in vivo*. J Tradit Complement Med. 6(4): 355-361.

Sharifi-Rad J, Salehi B, Stojanović-Radić ZZ, Fokou PVT, Sharifi-Rad M, Mahady GB, Sharifi-Rad M, Masjedi MR, Lawal TO, Ayatollahi SA, Masjedi J, Sharifi-Rad R, Setzer WN, Sharifi-Rad M, Kobarfard F, Rahman AU, Choudhary MI, Ata A and Iriti M. (2020) Medicinal plants used in the treatment of tuberculosis - Ethnobotanical and ethnopharmacological approaches. Biotechnol Adv. 44: 107629.

Snoek-van Beurden PAM and Von den Hoff JW. (2005) Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors. Biotechniques 38(1): 73-83.

Taneja NK and Tyagi JS. (2007) Resazurin reduction assays for screening of anti-tubercular compounds against dormant and actively growing *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG and *Mycobacterium smegmatis*. J Antimicrob Chemother. 60(2): 288-293.

Taprial S, Kashyap D, Mehta V, Kumar S and Kumar D. (2013) Antifertility effect of hydroalcoholic leaves extract of *Michelia champaca* L.: an ethnomedicine used by Bhatra women in Chhattisgarh state of India. J Ethnopharmacol. 147(3): 671-675.

Vijayalakshmi D, Dhandapani R, Jayaveni S, Jithendra PS, Rose C and Mandal AB. (2012) *In vitro* anti inflammatory activity of *Aloe vera* by down regulation of MMP-9 in peripheral blood mononuclear cells. J Ethnopharmacol. 141(1): 542-546.

Vimala R, Nagarajan S, Alam M, Susan T and Joy S. (1997) Antiinflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. Indian J Exp Biol. 35(12):1310-1314.