ANTIMICROBIAL ACTIVITIES OF SOLVENT EXTRACTS OF *Acacia auriculiformis*

A PROJECT REPORT

Submitted by

HARRISON C (20107008)

In partial fulfilment for the award of the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

Under the guidance of

Dr. R.ANITHA

Assistant professor, HITS

HINDUSTAN
INSTITUTE OF TECHNOLOGY AND SCIENCE

DEPARTMENT OF CHEMICAL ENGINEERING

OCT 2021

BONAFIDE CERTIFICATE

Certified that the project report **"ANTIMICROBIAL ACTIVITIES OF SOLVENT EXTRACTS OF *ACACIA AURICULIFORMIS*"** is the bonafide work of **HARRISON C (20107008)** who carried out the project work under my supervision.

SIGNATURE	**SIGNATURE**
HEAD OF THE DEPARTMENT	**SUPERVISOR**
Dr. B. VIVEKANANDAN	**Dr. R. ANITHA**
Associate Professor	Assistant Professor
Department of Chemical Engg.	Department of Chemical Engg.

INTERNAL EXAMINER	**EXTERNAL EXAMINER**
Name: Dr. R. Anitha	Name: Dr. R. Shivashankar
Designation: A.P (S.G)	Designation: A.P (S.G)

The project viva voce Examination is held on 11.10.2021

ACKNOWLEDGEMENT

This report has been prepared for the project that has been done for the analysis of antimicrobial property of the medicinal plant *Acacia auriculiformis* with the purpose of fulfilling the requirements of the course of BIOTECHNOLOGY.

At the outset we would like to extend our sincere gratitude to **Dr. (Mrs). ELIZABETH VERGHESE,** Chancellor of Hindustan Institute of Technology and Science for her endeavour in educating us in her esteemed institute which has helped us work towards our goal.

We also express our sincere thanks to **Dr. S. N. SRIDHARA,** Vice Chancellor of Hindustan Institute of Technology and Science and to **Mr. ASHOK VERGHESE,** Director of Hindustan Institute of Technology and Science.

I would also like to thank **Dr. B. VIVEKANANDAN,** Head of the Department for his constant support. I would like to extend my gratitude to **DR. R. ANITHA**, Assistant professor, Department of Chemical Engineering for guiding me throughout the project.

Abstract

Medicinal plant formed the basis and foundation stone of diseases from the very beginning of human civilization. Medicinal component from plants play many important roles in traditional medicine. People in all around the worlds have long been applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. It is estimated that there are about 2,500,000 species of higher plants and the majority of these have not been investigated in details for their pharmacological activities. In developing countries, about 80% of the population relies on traditional medicine for their primary health care needs.

Bacterial microbes are everywhere. Some are useful but many are harmful. Antibacterial activities present in *Acacia auriculiformis* inhibits the growth of bacteria in a beneficial manner. Also, the plant has antioxidant activities. First we got *Acacia auriculiformis* leaves and extracted it using methanol and water. The aqueous extract and methanol extract seem to have antibiotic and antioxidant activities. The aqueous extract exhibited better antibacterial activity against *E. coli* followed by *Bacillus sp.* and *Pseudomonas sp*. With further evaluation towards other biological and pharmacological activity of the tested plant material, it is possible to isolate druggable leads towards treatment of various ailments like infections, inflammations and etc.,

Keywords: *Acacia auriculiformis*, Antimicrobial, Antioxidant, Antibiotic and Sequential extraction.

TABLE OF CONTENTS

S.NO	CONTENT	PAGE NO.
1.	Introduction	6
2.	Literature review	10
3.	Materials and Methods	13
3.1.	Plant collection and extraction	13
3.2.	Antibacterial study	14
4	Results and discussion	15
4.1	Extraction	15
4.2	Antimicrobial study	16
5	Conclusions	17
6	References	18

1. INTRODUCTION

Medicinal plants have been discovered for medicinal purposes since prehistoric times. Plants synthesize hundreds of chemical compounds including defense against harmful organisms. Numerous phytochemicals with potential biological activity are identified. However since a whole plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Photochemical effects and pharmaceutical activities of many medicinal plants remain unassessed by rigorous scientific research to define efficacy and safety. *Acacia auriculiformis*, commonly known as auri, earleaf acacia, ear pod wattle, northern black wattle, Papuan wattle, and tan wattle, akashmoni in Bengali, is a fast-growing, crooked, gnarly tree in the family Fabaceae. It is native to Australia, Indonesia, and Papua New Guinea. It grows up to 30m tall. *Acacia auriculiformis* has about 47 000 seeds/kg. It grows 15 – 30 meters tall with a crooked bole that is up to 12 meters long and 50cm in diameter.

Although it produces leaves as a seedling, like most members of the genus the mature plant does not have true leaves but has leaf-like flattened stems called phyllodes. The wood is a very good fuel and is widely utilized for that purpose the plant is also harvested from the wild for local use as a medicine. The tree is commonly planted in tropical Asia, particularly as a fuel crop and to provide pulp for the paper industry; and is also sometimes planted in S. America and Africa. An excellent shade tree, it is planted to provide shelter on beaches and beachfronts. The population is currently believed to be stable and no major threats are known at present. The plant is classified as 'Least Concern' in the IUCN Red List of Threatened Species (2013).

The tree is predominantly found at low elevations in the seasonally dry tropical lowlands of the humid and sub-humid zones. It is usually found at elevations below 80 meters, but can be found up to 400 meters. In its natural range, the mean annual rainfall varies from (700 – 2,000) mm, and the dry season (i.e. Monthly rainfall less than 40 mm) can be up to 7 months. The mean maximum temperature of the hottest month is (32 – 34) °C, and the mean minimum of the coolest month is (17 – 22) °C. Frost does not occur in its natural range, but elsewhere, it tolerates light frost.

Requires a sunny position, it is very intolerant of shade. Found most commonly on clay soils, it exhibits the ability to grow in a wide variety of soils including calcareous sands and black cracking clays, poor-fertility soils, seasonally waterlogged soils, sandy loams and coral rag. It can also tolerate highly alkaline and saline soils, pH ranging between 4.3 and 9. It has brittle, easily-broken branches and therefore requires a position sheltered from strong winds. Established plants can tolerate periodic inundation and are also very drought tolerant. Often cultivated for timber outside its native range, the tree has escaped from cultivation in many parts of the tropics and has become an invasive weed in some areas. Very fast-growing, an increment in height of 2 – 4 meters per year in the first few years is common even on soils of low fertility.

The optimal planting density is not clearly established. Most current plantings are spaced at 2 – 4 x 2 – 4 meters, the closer spacing being more suitable for firewood and pulp plantations. The tree responds well to pollarding. Young trees respond to coppicing better than old trees, but the tree does not sprout vigorously or prolifically. Best results are obtained if the stump is cut at a height of 0.6 – 1 meter above the ground. Under favorable conditions, trees may reach a height of 15 meters in 5 years and produce an annual wood increment of 15-20 cubic m/ha over 10 – 12 years. Recommended rotation is 4 – 5 years for fuel wood, 8 – 10 years for pulp and 12 – 15 years for timber. Removal of lower branches and of young plants has been suggested as a means of improving stem form and of reducing the incidence of multiple stems. The tree has a shallow, spreading root system. Seedlings have the ability to compete with *Imperata cylindrica* during early growth phases and once mature may reduce the grass to a sparse ground cover. Hybridizes with A. *mangium*. The plant flowers and produces fruit throughout much of the year

This species has a symbiotic relationship with certain soil bacteria, these bacteria form nodules on the roots and fix atmospheric nitrogen. Some of this nitrogen is utilized by the growing plant but some can also be used by other plants growing nearby. It is being used traditionally to overcome various medical complications like sore eyes, aches, rheumatism, allergy, itching, and rashes. Besides, *Acacia auriculiformis* has been proven for many pharmacological activities like central nervous system depressant activity, antioxidant, antimicrobial, antimalarial, anti-filarial, cestocidal, anti-mutagenic, chemo-preventive, spermicidal, wound healing, hepato-protective and antidiabetic activity due to its low toxicity (LD50 = 3 741.7 mg/kg) and high efficacy. In addition, various phytochemical investigations reveal the presence of chief constituents as flavonoids (Auriculoside) and triterpenoid saponin glycosides (acaciasides- acaciaside A & B) in different parts of this plant. Since many years researchers have been carrying out various studies on this medicinal important shrub to elicit the various biological activities. This review attempts to highlight the pharmacognostical, phytochemical and pharmacological observations from 1965 to 2018 retrieved from SciFinder, Scientific journals, books, Google Scholar, and botanical electronic database websites. The various plant extracts evaluated for different pharmacological activities showed significant efficacy. Bioactive phytoconstituents isolated from various parts of the plant are highlighted. Pharmacognostical standardization of the plant done with various standard parameters is also reported. The low toxicity of this plant and the presence of major bioactive phyto-constituents like flavonoids and triterpenoid saponin glycosides are responsible for a therapeutic remedy for various diseases and pharmacological activities respectively.

In this project we're about to extract antimicrobial and antioxidant activities from *Acacia auriculiformis* through leaves samples of *A. auri.*

Uses of Auriculiformis

The tree is used as a folk medicine in Australia. A decoction of root is used to treat aches and sore eyes while an infusion of bark is used to treat rheumatism by aborigines of Australia. [4] Seeds are used to treat skin diseases like itching, allergy and rashes. The plant is useful as antimalarial remedy by the Ibibios of Niger Delta region of Nigeria.

Origin and geographic distribution

National Strands of Acacia auriculiformis are found in Australia (Cape York Peninsula, Queensland, Northern areas of northern territory), South West Papua New Guinea and Indonesia. The domestication of Acacia auriculiformis began 50 years ago. It is planted widely in Tropical Asia, with extensive planting in China andIndia. It has become naturalized in Western Malaysia. It is planted to a lesser extentin Africa and South America. In the present study, extraction of acacia auriculiformisis used for the identification of Antibacterial and antioxidant activities.

2. REVIEW OF LITERATURE

The evolution of human history shows the evidence that people are using traditional medicine for therapeutic purpose. The recent reports from the World Health Organization (WHO) claim that 70%- 80% population is primarily dependent on animals and plant-basedmedicines because of limited or no access to medical services. The drugs obtained from wild plants and animals are not only used as traditional medicines but also as raw materials in the formulation of modern allopathic and herbal preparations [1]. Acacia auriculiformis (A. auriculiformis) A.Cunn. ex Benth. belonging to family Fabaceae, is a straight, medium-sized, deciduous or evergreen tree, potentially accomplishing 30 m tallness, and is normally found in the roadsides and parks of India. The generic name of acacia is derived from the Greek word 'akis' which means a spike or a point. Whereas the Latin word 'auricula' implies outside ear of creatures which is a particular name through the word 'forma' implying frame, figure or shape. The tree is native to Australia and was first introduced to India in 1946 in West Bengal [2]. The tree is rich in glucuronic acid, methylglucuronic acid, arabinose, rhamnose and galactose [3]. It is reported that plant has shown various pharmacological activities like antioxidant[4], antimicrobial[5], antimalarial[6], antifilarial[7], cestocidal[8], antimutagenic, chemopreventive[9], spermicidal[10], hepatoprotective[11], wound healing[12] and antidiabetic activity[11]. The central nervous system (CNS) depressant activity was observed in a flavan glycoside known as auriculosideisolated from A. auriculiformis [9,13,14]. The main objective of the review was to highlight the updated pharmacological and phytochemical investigations about A. auriculiformis plant till date. The most relevant and exhaustive literature search was made using keywords "A. auriculiformis pharmacology", "A. auriculiformis phytochemistry", "A. auriculiformis patents" in SciFinder, PubMed, Scopus, and Google Scholar databases. The literature search results revealed articles from 1965 to 2018. Thereafter pharmacological and phytochemical related specific articles were carefully screened, selected and reviewed without any chronological restriction for the compilation of the present manuscript.

The botanical name of A. auriculiformis is A. auriculiformis A.Cunn. ex Benth. and the vernacular names include akashmoni in Bengali, Australian wattle in English, Bengali babul in Hindi, minnumaan, kondamanu, seema babul, maha babul in Telugu, kaththi karuvel in Tamil, aurculis in Kannada, Australian babool, akashia in Marathi and other names include Dal Moth, Earleaf Acacia, Auri, Earpod Wattle,Wattle, Black Wattle, Tan Wattle, Northern Black Wattle, Darwin BlackWattle, and Papuan Wattle. The A. auriculiformis belongs to domain Eukaryota, kingdom Plantae, subkingdom Viridiplantae, infrakingdomStreptophyta, phylum spermatophyte, subphylum angiospermae, class magnoliopsida/dicotyledonae, subclass rosidae, superorder rosanae, order fabales, family fabaceae/leguminosae, division tracheophyte, subdivision spermatophytina, genus acacia mill, and species A. auriculiformis A.Cunn. ex Benth[15-17]. The synonyms of A. auriculiformis include A. auriculiformis A.Cunn. ex Benth., orth. Var., Acacia moniliformis Griseb. and Racosperma auriculiformae (A.Cunn. ex Benth.), Pedley[17,18]. The flowering and fruiting season of A. auriculiformis varies from region to region as in Australia flowering season occurs from April to July and ripened seeds are available after 4-5 months later i.e. in August October.

In Malaysia flowering is observed from February to May and ripened seeds from fruits can be collected from October to April. In Java, Indonesia flowering season lies from March to June while in Thailand mature seeds can be collected between August and February. In India, the flowering season of A. auriculiformis takes place in the months of December to January and fruiting in February to March. Whereas in some regions of India flowering and fruiting take place in the months of

March to December but more in September to October. The bark, leaves, and fruits (pods with seeds and funicles) parts of A. auriculiformis are widely used for various biological activities[19,20].

In Australia, A. auriculiformis tree is used as a folk medicine by the natives for various diseases. For the treatment of sore eyes and aches, a decoction of the root is used for rheumatism treatment and bark infusion is used by the Australian aborigines[21]. The seeds of the tree are also used as skin ailment in various diseases like itching, allergy, and rashes[22]. The Ibibio community of Niger Delta region in Nigeria uses this plant as antimalarial medicine[6]. The various parts of A. auriculiformis plant extract and phytoconstituents are found to be useful in various diseases like candidiasis, rheumatism, conjunctivitis, pain, anthelmintic, human immunodeficiency virus (HIV), and microbial diseases[23]. The pharmacognostical standardization is an important aspect to control the authenticity of plants. It helps to control adulteration and ensures the quality of plants to accept them for worldwide use in trade and medicine system. The morphological or macroscopic studies help in identification of visual aspects of plant properties like organoleptic characters (color, odor, taste), shape, size etc. Sharma et al., in 2017 reported pharmacognostical standardization studies of A. auriculiformis[24]. Some of the important characteristics are highlighted here. The height of the A. auriculiformis tree is 35 to 40 feet and it spreads up to 25 to 35 feet. The crown has irregular uniformity, round shape, and moderate density. It has a fast growth rate and mediumtexture. The leaves of the tree are of the green and simple type with the alternate arrangement. The leaves have an entire margin with linear shape and parallel venation.

3. MATERIALS AND METHODS

3.1 Plant sample and extraction protocol

The plant parts of *Acacia auriculiformis* above ground were collected from the university campus of Hindustan University, Tamil Nadu, India. The plant material was shade-dried and milled and was subjected to extraction using water and methanol. The crudely powdered drug (20 g) was placed in an amber bottle with hexane (200 ml) and kept at ambient temperature for a period of a minimum of 48 hrs with repeated agitation till the resolvable substance has dissolved. The blend was squeezed, the marc (the damp solid matter) was hard-pressed and the collective liquids were cleared up by filtration. The filtrate was concentrated using evaporation at room temperature and later fractionated successively. Powdered form of Acacia is obtained by air drying the leaves in sunlight to get rid of moisture followed by grinding. One fine set of powdered acacia is taken with methanol for extract in conical flask whereas the other set is taken up with water in another flask. Using What man no 1 filter paper, the contents are filtered to obtain methanol and aqueous extract. These extracts are plated in Petri plates and dried in sunlight for 24 hours. The powdered extracts are dissolved in methanol and kept refrigerated for another 24 hours in Falcon tubes.

3.2 Determination of antimicrobial activity

Microorganisms and culture conditions: Three Gram-positive strains namely, *Bacillus sp.*, *E. coli* and *Pseudomonas sp.* were used in the present study. All the test organisms were maintained on Muller-Hinton agar (MHA) plates and stored at 4 °C, sub-cultured once a week. Potato dextrose agar (PDA) was used to culture fungal strains.

3.2.1 Antimicrobial susceptibility test (AST)

Antimicrobial activity was predicted by the agar well diffusion method. The MHA plates were inoculated with the prepared inoculum and a hole with a width of 6 mm was punched using a sterile cork borer. 50 µl of test extracts (12.5 mg) were positioned onto each well. Ciprofloxacin 5 µg per disc was used as reference standard. After 24 hrs of incubation at 37 °C, the existence of a clearing zone around the wells was noticed and which indicated antimicrobial activity (zone of inhibition (ZOI)). The minimum inhibitory concentration (MIC) was determined by the broth microdilution method in Muller-Hinton broth using 10^5 cells/ml with extract concentration of 0-1000 µg/ml. Ciprofloxacin and 10 % DMSO were used as positive and negative controls respectively. The readings were taken in triplicates.

4. RESULTS AND DISCUSSION

4.1 Extraction and yield

The extraction yield obtained for each extract is given. The higher variation among aqueous and solvent extracts might be ascribed mainly to solvent polarity and the different obtainability of extractable components, resulting from the different chemical compositions of extracts. Aqueous extract showed more yield when compared to that of methanol extract.

4.2 Antimicrobial activity

Based upon the activity, ethyl acetate and ethanol extracts were chosen for further antimicrobial and anti-inflammatory study. The current study was performed to assess the antimicrobial potential of *Acacia auriculiformis* against certain human pathogens. The extracts displayed a varying degree of microbicidal activity against all the organisms tested.

In the current study, the aqueous extract displayed significant antibacterial activity against *E. coli* whereas methanol extract was effective against *Bacillus sp.* The ZOI values obtained for aqueous extract were ranged from a minimum of 15 ± 0.3 mm to a maximum of 39 ± 0.6 mm (*Pseudomonas aeruginosa*). While the ethanol extract varied from 18 ± 1.5 mm (*C. albicans*) to 39 ± 2.02 mm (*Streptococcus sp.*) of *C. auriculata*. Guruprasad and Reddy, (2015) demonstrated better antibacterial activity of methanol and chloroform extract of *A. auriculiformis* leaf against *B. subtilis*, *E. coli*, and *S. aureus*. Our ZOI values for the above extracts against the same organism were 1.5, 1.8, and 2.7 folds respectively higher than that of the one which is presented earlier for *C.auriculata* methanol extract. The least susceptible strains were *B. subtilis*, *E. coli*, *S. typhi*, and a fungal strain *C. albicans* for both the extracts.

A comparable tendency in terms of ZOI and MIC was observed in the previously reported study of *C. obtusifolia*, and *Senna sophora* leaf extracts (Surekha and Shankar, 2016). Based on the reports presented by Salvat et al., (2004) the plant extracts with MIC values less than or equal to 500 µg/ml designate a good anti-infective effect. Accordingly, our results offered herein exhibited reasonable antibacterial activity against test microbes. The fungicidal effect of both the extracts was tested against *C. albicans*, it showed the least ZOI of 15 and 18 mm for ethyl acetate (MIC: 500 µg/ml for both extracts), and ethanol extracts respectively. Till now there is no reports or data presented on the fungicidal effects of *C. auriculata* aerial parts, however, Timothy et al., (2012) similarly reported a significant antifungal activity of water and ethanolic leaf extracts of *C. alata* Linn. The anticancer potential of fungal species is yet another significant arena and the fungal-derived materials, capsular polysaccharides, proteins and other structures have been exploited in pharmaceutical industries currently. Also, several active compounds sequestered from fungal species have been expressed into nanoparticles in order to achieve more anticancer activity (How et al., 2021).

5. CONCLUSIONS

From the present study, the aqueous extract displayed better antimicrobial activity against *E. coli* and *Bacillus sp.* The plant has to be exploited more in the future for its other pharmacological properties. Whole depiction of the current study specified that both ethyl acetate and ethanol extracts exhibited high potential of anti-inflammatory and antioxidant activities. The study validates the therapeutic potential of *A. auriculiformis* signifying that the plant possibly will "lead" for the separation of new compounds with good competence to treat various diseases and disorders. The outcomes of the present study confirmed the use of this plant in traditional claims in managing diabetics and infections. Novel antibiotics and anti-diabetic drugs could be formulated from A. auriculiformis by further isolation and purification of active compounds for phytotherapy.

6. REFERENCES

Alves, M.J., Ferreira, I.C., Froufe, H.J., Abreu, R.M., Martins, A., Pintado, M., 2013. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J. Appl. Microbiol. 115, 346–357.

Ashok, J.P., Harish, P.H., Prasad, W.V., Ashok, W.A., 2018. Comparative assessment of antioxidant potential of *A. auriculata* flower, leaf and seed methanolic extracts. Int. J. Pharm. Pharm. Sci. 7, 381-385.

Bora, R., Khakhalar, S., Dutta, T., 2019. Phytochemical profiling, assessment of total henolic content, total flavonoid content, and antioxidant activity of ethno-medicinal plant, *Meyna spinosa* from Assam. Asian J. Pharm. Clin. Res. 12, 61-63.

Chai, W.S., Sun, D., Cheah, K.H., Li, G., Meng, H., 2020. Co-electrolysis-assisted decomposition of hydroxylammonium nitrate−fuel mixtures using stainless steel−platinum electrodes. ACS Omega 5, 19525−19532.

Chai, W.S., Cheah, K.H., Meng, H., Li, G., 2019. Experimental and analytical study on electrolytic decomposition of HAN-water solution using graphite electrodes. J. Mol. Liq. 293, 1-9.

David, A.V., Arulmoli, R., Parasuraman, S., 2016. Overviews of biological importance of quercetin: a bioactive flavonoid. *Pharmacogn. Rev*. 10, 84–89.

Guruprasad, C., Nille Reddy, K.R.C., 2015. A Phyto-pharmacological Review of Plant – *Cassia auriculata.* Int. J. Pharm. Biol. Arch. 6, 1–9.

Habu, J.B., Ibeh, B.O., 2015. *In vitro* antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of new *bouldia laevis* ethanolic leaf extract. Biol. Res. 48, 1-10.

Han, M., Song, Y., Zhang, X., 2016. Quercetin suppresses the migration and invasion in human colon cancer caco-2 cells through regulating toll-like receptor 4/nuclear factor-kappa B pathway. Pharmacogn. Mag. 12, 237-244.

Hossain, M.A., Shah, M.D., Mahyar, S., 2011. Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremia borneensis*. Asian Pac. J. Trop. Med. 4, 637–664.

Jyothi, S.G., Sahana, C.S., Chavan Somashekaraiah, B.V., 2012. In vitro and in vivo antioxidant and antidiabetic efficacy of *Cassia auriculata* L. flowers. Global J. Pharmacol. 6, 33-40.

Karthikaiselvi, R., Subhashini, S., 2014. Study of adsorption properties and inhibition of mild steel corrosion in hydrochloric acid media by water soluble composite poly (vinyl alcohol-methoxy aniline). J. Assoc. Arab Uni. Basic Appl. Sci. 16, 74-82.

Karthikeyan, V., Baskaran, A., Rajasekaran, S., 2016. Gas chromatography-mass spectrometry (GC-MS) analysis of ethanolic extracts of *Barleria acuminata Nees*. Int. J. Pharm. Res. 6, 55-61.

Kooti, M., Farokhipour, M., Asadzadeh, Z., Larky, D.A., Samani, M.A., 2016. The role of medicinal plants in the treatment of diabetes: a systematic review. Electron. Physician. 8, 1832-1842.

Manikandan, R., Vijaya, A., Muthumani, G.D., 2013. Phytochemical and *in vitro* anti-diabetic activity of methanolic extract of *Psidium guajava* leaves. Int. J. Curr. Microbiol. Appl. Sci. 2, 15-19.

Moncada, A., Palmer, R.M., Higgs, E.A., 1991. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Nambirajan, G., Karunanidhi, K., Ganesan, A., Rajendran, R., Kandasamy, R., Elangovan, A., Thilagar, S., 2018. Evaluation of antidiabetic activity of bud and flower of *Avaram Senna* (*Cassia auriculata*) in high fat diet and streptozotocin induced diabetic rats. Biomed. Pharmacother. 108, 1495-1506.

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C.J., Valko, M., 2017. Targeting free radicals in oxidative stress related human diseases. Trends Pharmacol. Sci. 38, 592-607.

Ramma, A.L., Bahorun, T., Soobrattee, M.A., Aruoma, O.L., 2002. Antioxidant activities of phenolic, pro anthocyanidin, and flavonoid components in extracts of *cassia fistula*. J. Agril. Food Chem. 50, 5042−5047.

Rang, H.P., Ritter, J.M., Flower, R.J., Henderson, G. 2015. Rang & Dale’s Pharmacology, Elsevier: London.

Richard, R., Boschi, G., Chau, M.T., Cavier, R., 1963. Phenol-piperazine adducts showing anthelmintic properties. J. Med. Chem. 16, 725–728.

Salvat, A., Antonacci, L., Fortunato, R.H., Suarez, E.Y., Godoy, H.M., 2014. Antimicrobial activity in methanolic extracts of several plant species from northern Argentina. *Phytomed*. 11, 230–234.

Samarghandian, S., Farkhondeh, T., Nezhad, M.A., 2017. Protective effects of chrysin against drugs and toxic agents. Dose Res. 15, 1-10.

Stojkovic, D., Petrovi, C.J., Sokovi, C.M., Glamoclija, J., Kukic-Markovic, J., Petrovic, S., 2013. *In situ* antioxidant and antimicrobial activities of naturally occurring caffeic acid, *p*-coumaric acid and rutin, using food systems. J. Sci. Food Agril. 93, 3205–3208.

Surana, S.J., Gokhale, S.B., Jadhav, R.B., Sawant, R.L., Wadekar, J.B., 2008. Anti-hyperglycemic activity of various fractions of *Cassia auriculata* Linn. in alloxan diabetic rats. Indian J. Pharm. Sci.70, 227–229.

Surekha, R., Deshpande Shankar, N., 2016. Evaluation of *in vitro* antimicrobial activity of extracts from *Cassia obtusifolia* L. and *Senna sophera* (L.) Roxb against pathogenic organisms. J. Appl. Pharm. Sci. 6, 83-85.

Timothy, S.Y., Wazis, C.H., Adati, R.G., Maspalma, I.D., 2012. Antifungal activity of aqueous and ethanolic leaf extracts of *Cassia alata* Linn. J. Appl. Pharm. Sci. 2, 182-185.

Zhang, X., Jia, Y., Ma, Y., Cheng, G., Cai, S., 2018. Phenolic Composition, antioxidant properties, and inhibition toward digestive enzymes with molecular docking analysis of different fractions from *Prinsepia utilis* Royle fruits. Molecules 23, 33-73.