



Copyright © 2016 Kalavani *et al*

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORIGINAL RESEARCH

Evaluation of anti-inflammatory and antibacterial activity of *Pithecellobium dulce* (Benth) extract

R. KALAVANI¹, R. Sabitha BANU¹, K. A. JEYANTHI¹, T. Uma SANKARI², A. Vinoth KANNA^{1*}

¹Department of Biotechnology, Thanthai Hans Roever College, Elambalur, Perambalur – 621 212, Tamil Nadu, India

²Department of Molecular Biology, School of Life Sciences, Madurai Kamaraj University, Madurai – 625 027, Tamil Nadu, India

*Corresponding Author email: avinokanna@live.in

• Received: 20 July 2016 • Revised: 26 August 2016 • Accepted: 03 September 2016 • Published: 22 September 2016 •

ABSTRACT

The use of herbal plants and their extracts have been utilized by all over the world for its various biological activities. The use of ethanolic *Pithecellobium dulce* extract has been analyzed for its anti-inflammatory and antibacterial activities. For this, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* have been studied against the *P. dulce* extract. The result which shows the presence of many difference secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, steroids, tannins, terpenoids and saponins have been detected. The anti-inflammatory responses such as percentage of inhibition of protein denaturation and HRBC membrane stabilization has showed an increase response when compared with the standard drug Aspirin of about 62.80 and 59.25% respectively. Also the antibacterial response showed a positive response when compared with the standard drug gentamycin at the lower concentration. Hence, the ethanolic extract of *P. dulce* can be used as a potential agent as anti-inflammatory and anti-bacterial compounds.

KEY WORDS: *Pithecellobium dulce*, protein denaturation, HRBC, anti-bacterial agent

Introduction

Health is the level of functional or metabolic efficiency of a living organism. In humans, it is the ability of individuals or communities to adapt and self-manage when facing physical, mental or social challenges (Huber *et al.*, 2011). Inflammation is a part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells or irritants (Ferrero-Miliani *et al.*, 2007). Inflammatory abnormalities are a large group of disorders that underlie a vast variety of human diseases. The immune system is often involved with inflammatory disorders, demonstrated in both allergic reactions and some sympathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with etiological origins in inflammatory processes include cancer, atherosclerosis, and ischemic heart disease

(Kumar *et al.*, 2011).

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation or swelling. Several drugs have been utilized against the inflammation and in specific to this the drug aspirin has utilized by the predominant people's all over the world. Long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) can cause gastric erosions, which can become stomach ulcers and in extreme cases can cause severe hemorrhage, resulting in death (Bandolier, 2012).

Plants have a healing potential and may have antimicrobial characteristics was well accepted since long before. Among the estimated wide range of medicinal plants (250,000-500,000 plant species) only a limited percentage of plants have been explored. Medicinal plant plays an important role in the development of new drug. Natural product could be

potential drug for human and livestock and their analogues can act as an intermediate for the synthesis of useful drugs. Plant possess many phytoconstituents with various biological activity including antioxidant, ant diabetic, anticancer etc., (Preethi and Mary Saral, 2014). The use of herbal medicines has been steadily increasing over the past decade. A considerable number of these plants/plant based products have been widely used. There are 2,50,000 to 5,00,000 species of plants on earth (Cowan, 1999) out of which only 1-10% are used as food for both humans and other animal species, however more than these are used as medicinal purpose (Devi *et al.*, 2009). Use of herbal plants as drugs in our Ayurveda, Homeopathic, Unani and Chinese system is as old as civilization, 60% of the world's population exclusively rely on traditional medicine (plant extract) for their primary healthcare needs (Fransworth, 1994).

Pithecellobium species (leguminosae) are widely distributed in the tropics, chiefly in Asia and America. *Pithecellobium dulce* Benth, a most versatile medicinal plant, has attracted a worldwide prominence in recent years, owing to its wide range of medicinal properties and diverse utility. All plant parts of the *P. dulce* elaborates a vast array of biologically active compounds and have been demonstrated to exhibit anti-diabetic, locomotors, anti-venom, free radical scavenging, protease inhibitor, anti-inflammatory, anti-bacterial, anti-mycobacterium, Abortifacient, spermicidal, anti-convulsant, anti-ulcer, anti-diarrheal, anti-fungal, anti-tubercular, anti-tumor and anti-oxidative properties. Here the compounds present in different parts of *P. dulce* and biological activities of their extracts or the chemical constituents as reported in the literature since 1962 to 2013 have been reviewed (Shweta and Mehta, 2013).

In the present study, the ethanolic extract of *P. dulce*, have been utilized for its albumin degradation and membrane stabilization assays and also the for its antibacterial activity against the human's pathogens.

Materials and Methods

Collection of plant material

The *P. dulce* plant was identified collected from Perambalur areas. The plant material was washed thoroughly, initially with tap water and then with distilled water to remove any debris or dust particles and was then allowed to dry in an oven at 40°C. The dried plant material was ground to a fine

powder and stored at room temperature in airtight containers until used further.

Preparation of plant extract

To 500g of *Pithecellobium dulce* leaf powder, 1500 ml of ethanol was added for the extraction as the solvent for 24h at room temperature, after which the supernatant of each solvent was recovered by filtering through Whatmann filter paper. This process was repeated thrice and the respective solvent from the supernatant was evaporated in a rotary vacuum evaporator to obtain the crude extract. These extracts were stored at 4°C until used for the evaluation of anti-microbial activity.

Phytochemical Screening

Phytochemical test was carried out on ethanolic extracts of *P. dulce*, using standard procedures to identify the constituents such as alkaloids, fatty acids, flavonoids, glycosies, phenols, resins, saponins, steroids, tannins, terpenoids, proteins, sugars and anthraquinones (Harbone, 1973).

In vitro anti-inflammatory activity of plant extracts

Inhibition of albumin denaturation

The anti-inflammatory activity of plant extract was studied by using inhibition of albumin denaturation technique which was studied Sakat *et al.* (2010) followed with minor modifications. The reaction mixture (0.5 ml; pH 6.3) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of distilled water. pH was adjusted at 6.3 using a small amount of 1 N HCl. Different concentrations of plant extract were added to the reaction mixture and were incubated at 37°C for 20 min and then heated at 57°C for 5 min after cooling the samples, 2.5 mL of phosphate buffer saline was added. Turbidity was measured spectrophotometrically at 600 nm. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage Inhibition (\%)} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{Control}} \times 100$$

HRBC membrane stabilization method

Blood was collected (2 mL) from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution and centrifuged at 3000 rpm. The packed cells were washed with isosaline solution and a 10 % v/v suspension was prepared with normal saline and kept at 4°C undisturbed before use. Different concentrations of plant extract (50, 100, 200, 500 and 1000 µg /0.5 ml) in normal saline, Aspirin as standard

(50, 100, 200, 500 and 1000 µg / 0.5 ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of 10% HRBC suspension was added to prepared. All the assay mixtures were incubated at 37° C for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

$$\text{Percentage stabilization} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{Control}} \times 100$$

Statistical Analysis

Data obtained from this study were expressed as mean ± SD. Statistical analysis was performed using SPSS version - 17, were considered statistically significant.

Anti-bacterial Activity

Ethanollic leaf extracts *Pithecellobium dulce* were tested by the well diffusion method (Jain, 2009; Joshi *et al.*, 2011) against gram positive bacteria's *S. pyrogenes* and *S. aureus* and gram negative bacteria's *E. Coli* and *K. pneumonia*. Different concentration of the extracts (100µg/ml) was prepared by reconstituting with ethanol. The test microorganisms were seeded into respective medium by spread plate method 10µl (10 cells/ml) with the 24hr cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Gentamycin (10µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24hrs. The diameters of the inhibition zones were measured in mm.

Results and Discussion

Anti-inflammatory activity

Inflammation is a common phenomenon and it is a reaction of living issue towards injury. The present study involved a preliminary phytochemical analysis of the crude extracts, the results of which have been shown in Table-1. Preliminary phytochemical analysis of leaf extracts showed the presence of alkaloids, tannin, saponins, glycosides, anthraquinones, terpenoids and sterol etc.

Several flavonoids isolated from medicinal plants have been discovered to possess significant anti-inflammatory effects

(Duke, 1992). According to Oktay *et al.* (2003) a highly positive relationship between total phenolics and antioxidant activity appears to be the trend in many plants. The anti-inflammatory activity is a common property of many flavonoids, tannins and sterols (Goncalves *et al.*, 2008; Singh *et al.*, 1997; Vimala *et al.*, 1997) and these phyto constituents also possess antioxidant property (Oktay *et al.*, 2003). The presence of the said constituents in the extracts of *P. dulce* may be responsible for the observed activities.

Table 1: Qualitative analysis of ethanolic leaf extract of *Pithecellobium dulce*

Phytochemicals constituents	Ethanolic leaf extract of <i>Pithecellobium dulce</i>
Alkaloids	++
Glycosides	++
Phenol	++
Flavonoids	++
Steroids	++
Tannins	++
Terpenoids	++
Saponins	+

'+' Minimum presence; '++' Moderate presence; '+++' Maximum presence and '-' absence

In summary, our results suggest that *Pithecellobium dulce* has considerable potency in anti-inflammatory action and has prominent effects by *in-vitro* anti-arthritis assay protein denaturation method. Hence, the results of the present study support the traditional use of *P. dulce* in the treatment of rheumatism.

Protein Denaturation method

The production of auto antigen in certain arthritic disease may be due to denaturation of protein. Percentage inhibition of ethanolic extract was found to be 62.80% at 500µg/ml and the effect was compared with standard drug (Aspirin) (Figure 1). The results emphasize that the ethanolic extract was capable of controlling the production of auto antigen and inhibits denaturation of protein.

Despite the progresses in modern medicine, it has been reported that more than 70% of the developing world's population still depends on complementary and alternative systems of medicine, otherwise known as traditional medicine. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy

and cost effectiveness. In India there are several indigenous medicinal plants available that have anti-inflammatory capabilities.

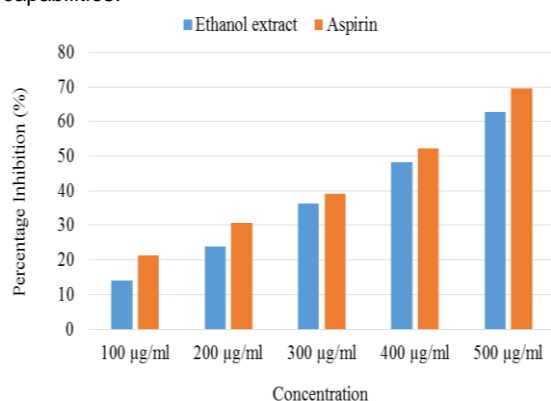


Figure 1: Protein denaturation assay of the ethanolic extract of *Pithecellobium dulce* compared with the standard drug Aspirin

In vitro anti-inflammatory activity was evaluated using protease enzyme inhibition method (Alam *et al.*, 2011). In a study (Rashid *et al.*, 2011), researchers revealed the significant *in vitro* membrane stabilizing effect of two Bangladeshi medicinal plants namely *Mesua nagassarium* and *Kigelia pinnata*, which indicates the anti-inflammatory activity of the medicinal plants. From *Persicari astagnina* (Ahmed *et al.*, 1997), *Scoparia dulcis* (Ahmed *et al.*, 2001) and *Sidacordi folia* (Sutradhar *et al.*, 2007) researchers isolated potent anti-inflammatory compounds and tested using standard methods. The compounds were of sesquiterpene, diterpene, and flavonoid glycoside and alkaloid types. In case of the rest of the medicinal plants the researchers conducted the anti-inflammatory study using the crude extracts and found significant activity.

In-vitro anti-inflammatory activity was evaluated by using albumin denaturation assay and membrane stabilization assay. Ethanolic extracts of *P. dulce* leaves showed significant results in *in-vitro* anti-inflammatory model. The results of *in-vitro* models showed that *P. dulce* has potential anti-inflammatory activity and acts through multiple mechanisms. This similar result showed in *in vitro* anti-inflammatory activity of the alcoholic extract of *Mimusops elengi* leaves (Khatri, *et al.*, 2014).

Inhibition of protein denaturation and human red blood cell membrane stabilization method was evaluated for anti-inflammatory activity. The maximum membrane stabilization of *P. dulce* was found at (73.85±0.80) % at a dose of

1000µg/0.5ml and that of protein denaturation was found to be 86.23% at a dose of 250µg/ml with regards to standards in anti-inflammatory activity (Kar *et al.*, 2012).

***In vitro* anti-inflammatory activity**

In this current research work *in vitro* anti-inflammatory activity was performed using HRBC Membrane stabilization method and protein denaturation method (Figure 2). HRBC is similar to lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane.

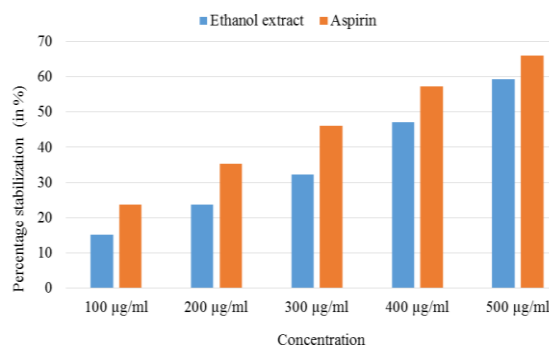


Figure 2: HRBC stabilization assay by the ethanolic extracts of *Pithecellobium dulce* leaves

HRBC membrane stabilization method

The *in vitro* anti-inflammatory method involves the stabilization involves stabilization of HRBC Membrane by hypotonicity induced membrane lysis. The percentage protection of ethanolic extracts was found 59.25 % at 500 µg/ml and the effect was compared with standard drug (Aspirin). This result may nearly similar to standard drug.

Similar studies on *in-vitro* anti-inflammatory activity of leaf extracts of *Basella alba*. Linn Varalba by Kumar *et al.* (2011) exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. Stabilization of lysosomal membrane is important in limiting the inflammation response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release. Gambhire *et al.* (2009) reported that methanol extract of *Murraya koenigi* leaves produces significant anti-inflammatory activities in dose dependent manner in membrane stabilization and inhibition of protein denaturation. Phytochemical screening indicates the presence of flavonoids, tannin, saponins, glycosides etc. In this current

research work, *in-vitro* anti-inflammatory and anti-arthritis activity were performed using HRBC membrane stabilization method and protein denaturation method. HRBC is similar to lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane. The membrane stabilizing activity of the extract may be due to the presence of flavonoids, alkaloids, tannins and or saponins present in *P. dulce*.

The crude extracts of the various parts or the whole plants of the medicinal plants and isolated compounds from the medicinal plants showed statistically significant anti-inflammatory activity both in *in vivo* and *in vitro* assay. *In vitro* anti-inflammatory activity was evaluated using protease enzyme inhibition method (Alam *et al.*, 2011). The ethanolic extracts of *Pithecellobium dulce* leaves preparation was initially subjected to erythrocyte (RBC) membrane

stabilization induced hemolysis by hypotonic solution. The erythrocyte membrane resembles to lysosomal membrane and as such the erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Omalev *et al.*, 2008). The vitality of cells depends on the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by hemolysis and oxidation of hemoglobin (Augusto *et al.*, 1982). An injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. It is therefore expected that phytochemicals present and their synergistic action as in *P. dulce* extracts with membrane stabilizing properties, should offer significant protection of the cell membrane against injurious substances (Liu *et al.*, 1992).

Table 2: Antibacterial activity *Pithecellobium dulce* leaf extracts

S. No	Pathogenic bacteria	Ethanol extract Zone of inhibition (mm)			Standard (Gentamycin) Zone of inhibition (mm)
		20 µl	40 µl	60 µl	
1.	<i>Streptococcus pyogenes</i>	06	09	11	08
2.	<i>Staphylococcus aureus</i>	06	08	12	08
3.	<i>Escherichia coli</i>	07	08	11	08
4.	<i>Klebsiella pneumoniae</i>	06	09	13	09

Antimicrobial Activity

Ethanolic extracts of the leaves of *P. dulce* were prepared and screened for their antibacterial activity against four different bacterial strains including both gram negative strains such as *E. coli* and *K. pneumoniae* and gram positive strains such as *S. pyogenes* and *S. aureus*. (Table 2). All the tested extracts exhibited significant antibacterial activity in a dose dependent manner. The results were compared with Gentamycin, a known antibiotic or antimicrobial agent. The results confirmed the antimicrobial potential of the plant and indicated that the acetone extract can be used in the inhibition of pathogenic bacteria. The similar result showed antimicrobial activity against all the three isolates. The ethanolic extracts prepared from bark of *M. elengi* were tested against *S. aureus*, *E. coli* and *P. aeruginosa*. The result (Figure 3) showed antimicrobial activity against all the four isolates. The ethanol extracts were found to be more potent than aqueous extracts of all the medicinal plants (Nair

and Chanda, 2007). In this study, ethanolic extract of the *P. dulce* showed minimum inhibitory concentration (MIC) at 60µl concentration shows 12mm zone of inhibition against *S. aureus*, 11mm zone of inhibition against *S. pyogenes*, 11mm zone of inhibition against *E. coli* and 13mm zone of inhibition against *K. pneumoniae*.

According to these findings, it can be suggested that, ethanol extract of *P. dulce* is more efficient than 20µl and 40µl concentration ethanolic extract against four bacterial strains. Briefly, the maximum antibacterial activity was observed against *K. pneumoniae* and the minimum antibacterial activity was observed against *S. pyogenes* and *E. coli*. In addition, *K. pneumoniae* is more susceptible than other test microorganisms to ethanol extract of *P. dulce*. To check the antimicrobial activity of *Azadirachta indica*, *Ocimum sanctum*, *Mimusops elelengi*, *Tinospora cordifolia* and Chlorhexidine Gluconate on common endodontic pathogens like *S. mutans*, *E. faecalis* and *S. aureus*. All the plants

extracts showed considerable antimicrobial activity against selected endodontic pathogens. *O. sanctum* was the most effective against *S. mutans*, *M. elengi* showed highest zone of inhibition against *E. faecalis*, whereas Chlorhexidine Gluconate was the most effective agent against *S. aureus* (Mistry *et al.*, 2014).

In another report, different solvent extracts of bark, fruits (fleshy portion) and leaves of *M. elengi* were screened for their antibacterial activity against some pathogenic bacteria and. The activities of the extracts were not significantly enough against most of the tested organisms. Fruit extracts were less potent against most of the tested organisms compared to those obtained from bark and leaves (Ali *et al.*, 2008). The aqueous and ethanol extract of ten medicinal

plants including leaf of *M. elengi* were tested against *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *B. cereus*, *A. fecalis* and *S. typhimurium*. The hexane, ethyl acetate, ethanol and methanol extracts showed antibacterial activity against *S. mutans* (Jebashree *et al.*, 2011). Petroleum ether, dichloromethane, ethyl acetate and ethanol extracts of seeds of *M. elengi* were tested for antibacterial efficacy against *E. coli*, *B. subtilis* and *S. typhi*. The compounds showed strong inhibitory activity against Gram positive and Gram negative bacteria (Hazra *et al.*, 2007). In agar well diffusion assay, the ethanolic extracts of plant showed considerable activity against all bacteria. The results confirmed the antimicrobial potential of the plant and indicated that the acetone extract can be used in the inhibition of pathogenic bacteria.

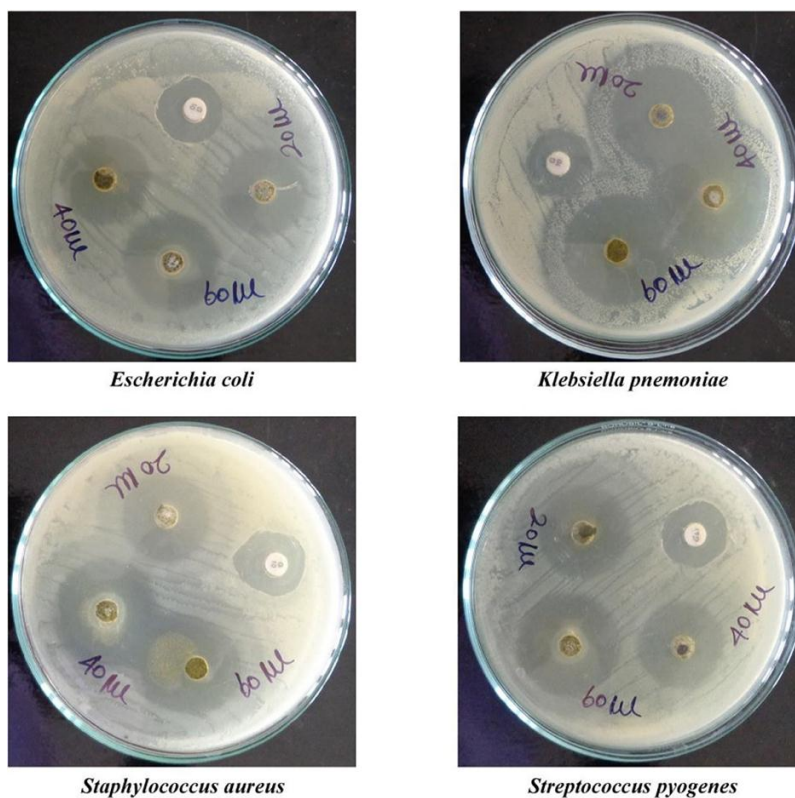


Figure 3: Antibacterial activity of ethanolic extract of *Pithecellobium dulce* leaves

Acknowledgement

We would like to thank The principal and management of Thanthai Hans Roever College, Perambalur for their moral and laboratory facilities support which helps to do our work in a better way. Also we express our gratitude to our Life science faculty's for their moral support to do this work in a successful way.

References

Ahmed, M., Sadhu, S. K., Datta, B. K., Kundu, J. K. and Bachar, S. C. 1997. Preliminary studies on the antiinflammatory, analgesic and diuretic activity of stagninol, a sesquiterpene isolated from *Persicaria stagnina*. *Die Pharmazie*. 52(6): 472-475.

Ahmed, M., Shikha, H. A., Sadhu, S. K., Rahman, M. T. and Datta, B. K. 2001. Analgesic, diuretic, and anti-inflammatory principle from *Scoparia dulcis*. *Die Pharmazie*. 56(8): 657-660.

Alam, M. B., Hossain, M. S. and Haque, M. E. 2011. Antioxidant and anti-inflammatory activities of the leaf extract of *Brassica nigra*.

- International Journal of Pharmaceutical Sciences and Research.* 2(2): 303-310.
- Ali, M. A., Mozd, M. A., Yeasmin, S., Khan, A. M. and Sayeed, M. A. 2008. An Evaluation of Antimicrobial Activities of *Mimusops elengi* Linn. *Research Journal of Agriculture and Biological Sciences.* 4(6): 871-874.
- Augusto, O., Kunze, K. L. and Montellano, P. R. 1982. N-phenylprotoporphyrin formation in the haemoglobin phenylhydrazine reaction. *Journal of Biological Chemistry.* 257(11): 6231-6241.
- Cowan, M. M. 1999. Plants products as antimicrobial agents. *Clinical Microbiology Reviews.* 12(4): 564-582.
- Devi, K., Karthikai, G., Thirumaran, G., Arumugam, R. and Anantharaman, P. 2009. Antibacterial activity of selected medicinal plants from Parangipettai Coastal Regions; Southeast Coast of India. *World Applied Science Journal.* 7(9): 1212-1215.
- Duke, J. A. 1992. Handbook of biologically active phytochemicals and their activities. CRC Press, Boca Raton, FL.
- Farnsworth, N. R. 1994. Ethnopharmacology and drug development. In: Ciba Foundation Symposium. 185:42-59.
- Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S. and Girardin, S. E. 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 β generation. *Clinical and Experimental Immunology.* 147(2): 227-235.
- Gambhire, M. N., Juvekar, A. R. and Sakat, S. S. 2009. Evaluation of anti-inflammatory activity of methanol extract of *Murraya koenigii* leaves by *in vivo* and *in vitro* methods. *Pharmacology online.* 1(10): 1072-1094.
- Goncalves, J. C. R., Oliveira, F. D. S., Benedito, R. B., Sousa, D. P. D. and Almeida, R. N. D. 2008. Anti-nociceptive activity of (-)-carvone: evidence of association with decreased peripheral nerve excitability. *Biological and Pharmaceutical Bulletin.* 31: 1017–1020.
- Harborne, J. B. 1973. *Phytochemical Methods.* Chapman and hall Ltd., London: U.K. 49-188.
- Hazra, K. M., Roy, R. N., Sen, S. K. and Laskar, S. 2007. Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengi* Linn. *African Journal of Biotechnology.* 6(12): 1446-1449.
- Huber, M., Knotnerus, J.A., Green, L., van der Horst, H., Jadad A.R., Kromhout, D. and Smid, H. 2011. How should we define health? *British Medical Journal.* 343: d4163.
- Jain, P., Bansal, D. and Bhasin, P. 2009. Antibacterial activity of aqueous plant extracts against *Escherichia coli* and *Bacillus subtilis*. *Drug Invention Today,* 2(4): 220-222.
- Jebashree, H. S., Kingsley, S. J., Sathish, E. S. and Devapriya, D. 2011. Antimicrobial activity of few medicinal plants against clinically isolated Human Cariogenic Pathogens- an *in vitro* Study. *ISRN Dentistry.* 6.
- Joshi, B., Sah, G.P., Basnet, B.B., Bhatt, M., Sharma, D., Subedi, K., Pandey, J., Malla, R. 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *Journal of Microbiology and Antimicrobials.* 3(1): 1-7.
- Kar, B., Kumar, R. B. S., Karmakar, I., Dolai, N., Bala, A. and Mazumder, U. K. 2012. Antioxidant and *in vitro* anti-inflammatory activities of *Mimusops elengi* leaves. *Asian Pacific Journal of Tropical Biomedicine.* 5: 976-980.
- Khatri, D., Manjulakonka, K. and Juvekar, A. R. 2014. Evaluation of anti-inflammatory activity of *Mimusops elengi* extracts indifferent *in vitro* and *in vivo* models. *International Journal of Pharmaceutical Biology and Science.* 5(1):259-268.
- Kumar, V., Bhat, Z. A., Kumar, D., Bohra, P. and Sheela, S. 2011. *In vitro* anti-inflammatory activity of leaf extracts of *Basella alba* Linn. var. Alba. *International Journal of Drug Delivery and Research.* 3(2): 176-179.
- Liu, G. T., Zhang, T. M., Wang, B. E. and Wang, Y. H. 1992. Protective action of seven natural phenolic compounds against peroxidative damage to biomembranes. *Biochemical Pharmacology.* 43(2): 147-152.
- Mistry, K. S., Sanghvi, Z., Parmar, G. and Shah, S. 2014. The antimicrobial activity of *Azadirachta indica*, *Mimusops elengi*, *Tinospora cordifolia*, *Ocimum sanctum* and 2% chlorhexidine gluconate on common endodontic pathogens: An *in vitro* study. *European Journal of Dental.* 8(2):172-177.
- Nair, R. and Chanda, S. V. 2007. Antibacterial activities of some medicinal plants of western region of India. *Turkish Journal of Biology.* 31:231-236.
- Okta, M., Gulcin, I., Kufrevioglu, O. I., 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extract. *Lebensm. -Wiss. Technology.* 36: 263–271.
- Preethi, S. and Mary Saral, A. 2014. GC-MS analysis of microwave assisted ethanolic extract of *Pithecellobium dulce*. *Malaya Journal of Biosciences.* 1(4): 242-247.
- Rashid, M. A., Sikder, M. A. A., Kaisar, M. A., Miah, M. K., Parvez, M. M. and Hossain A. K. M. N. 2011. Membrane stabilizing activity - a possible mechanism of action for the anti-inflammatory activity of two Bangladeshi medicinal plants: *Mesua nagassarium* (Burm. F.) and *Kigeliapinnata* (Jack) DC. *International Journal of Pharmaceutical Research and Development.* 3(3): 1-5.
- Sakat, S., Juvekar, A. R. and Gambhire, M. N. 2010. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharma and Pharmacological Sciences.* 2(1): 146-155.
- Shweta, S. and Mehta, B. 2013. A review on pharmacological activities of *Pithecellobium dulce* extract and their effective doses. *Journal of Medical Pharmaceutical and Allied Sciences.* 5: 37-45.
- Singh, S., Bani, S., Singh, G.B., Gupta, B. D., Banerjee, S. K. and Singh, B. 1997. Anti-inflammatory activity of lupeol. *Fitoterapia.* 68: 9-16.
- Sutradhar, R. K., Rahman, A. M., Ahmad, M., Bachar, S. C., Saha, A. and Guha, K. S. 2007. Anti-inflammatory and analgesic alkaloid from *Sida cordifolia* Linn. *Iranian Journal of Pharmacology and Therapeutics.* 20(3): 185-188.
- Vimala, R., Nagarajan, S., Alam, M., Susan, T. and Joy, S. 1997. Anti-inflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. *Indian Journal of Experimental Biology.* 35: 1310-1314.