Chapter 2

Review of Literature
2. REVIEW OF LITERATURE

2.1 Review of Plant

1. Panneerselvam CK et al.\textsuperscript{[155]} (2013) studied adulticidal, repellent, and ovicidal properties of indigenous plant extracts against the malarial vector, anopheles stephensi (Diptera: Culicidae). Among all other plants they found mortality of 100 % with methanol extract of \textit{A. paniculata} exerted at 150 ppm and aqueous, methanol extract of \textit{C. occidentalis} and \textit{E. hirta} were exerted at 300 ppm. These results suggest that the leaf extracts of \textit{A. paniculata}, \textit{C. occidentalis}, and \textit{E. hirta} have the potential to be used as an ideal eco-friendly approach for the control of the \textit{A. stephensi}.

2. Kumar S et al.\textsuperscript{[156]} (2011) evaluated 15 local plants collected from New Delhi, India, for its larvicidal activity against an Indian strain of dengue fever mosquito, \textit{Aedes aegypti} L. one of which was leaves of \textit{C. Occidentalis}. The hexane extracts of leaves of \textit{C. occidentalis} was found to have significant larvicidal potential with LC50 values ranging from 55.00 to 74.67ppm.

3. Mohammed M. et al.\textsuperscript{[157]} (2012) revealed that \textit{Cassia occidentalis} L. (Caesalpiniaeae) was exhaustively extracted with n-hexane and subsequently with methanol. The methanol portion was subsequently partition with chloroform, ethyl acetate and n-butannol. The phytochemical studies of the partition portion were done using standard protocols. The zone of inhibition (ZI), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. The antimicrobial screening revealed that the extract exhibited varying activity against different microbes. These activities observed could be attributed to the presence active metabolites contained in the extract.
4. Sundaram RL et al. [158] (2012) evaluated effect of aqueous extract of Cassia occidentalis on pentylenetetrazole-induced oxidative stress and kindling in rats using phenytoin as reference standard. The secondary metabolites in the aqueous extract of Cassia occidentalis were also quantified by UV spectrophotometer in which found 28.59% of phenol and 0.34% flavanoids. The IC50 of aqueous extract was 147.73 µg/ml and 100 mg/kg oral administration of the extract decreased the pentylenetetrazole kindled seizures and oxidative stress. Cassia occidentalis treated rats exhibited a tendency to prevent the impairment in the neurotransmitters as compared to untreated rats. The findings suggest potential used of aqueous extract of Cassia occidentalis as an effective agent to prevent oxidative stress mediated epilepsy.

5. Sadiq IS et al. [159] (2012) studied ethanol and water extract of Cassia occidentalis for In vitro antimicrobial activity on Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Shigella spp. Culin Chromatographic separation using glass column packed with wet silica gel using ethanol and methanol in ratio of 50:50 as mobile phase was carried out on ethanol and water extracts, and the efficacy of the resulting fractions was tested against the susceptible organism. Some of the extracts indicated significant inhibitory activity against the tested organisms. General phytochemical screening was done on the ethanol, water extracts and fractions. Ethanol and water extract revealed the presence of tannins, saponins, cardiac glycoside, terpenoids and anthraquinones while the fractions revealed the presence of tannins, terpenoid and anthraquinones. The result of antimicrobial screening show that the ethanol and water extracts inhibited the growth of various species of gram-negative bacteria. The ethanol extract of Cassia occidentalis leaves show highest activity against Salmonella typhi while the lowest activity was shown by the water extract against Shigella spp. This result might explain the ethnobotanical use of the plant for the treatment of dysentery, gastro internal disorder, constipation and typhoid fever.
6. Sathya A et al.\cite{160} (2012) studied phytochemistry of *Cassia occidentalis* and evaluate aqueous extract of *C. occidentalis* seeds for its antimicrobial and anti oxidant activity. Phytochemical analysis of aqueous extract showed the presence of anthraquinones, carbohydrates, glycosides, cardiac glycosides, amino acid, phytosterols, fixed oils and fats, phenolic compounds, tannins, flavonoids, steroids and saponins. They also estimated quantitatively phenol, flavanoids, carotenoids and heavy metals like cadmium, chromium, lead, mercury and nickel using different instruments. They found 3.24 µg/gm of flavanoids, 2.9 µg/gm of Carotenoids and 6.7 µg/gm of phenolics in plant. The antibacterial activity of *Cassia occidentalis* seed and leaf extract were tested by the agar diffusion method. For the determination of antibacterial activity, the antibiotic resistant bacteria namely, *Staphylococcus sp*, *Escherichia coli* and *Pseudomonas aeruginosa* were used. Aqueous extract was found to have maximum zone of inhibition against Escherichia coli, Pseudomonas spp, and Staphylococcus sp. The results demonstrated use of *Cassia occidentalis* aqueous plant extract as antimicrobial agent, antioxidant and used as herbal medicine for curing number of disease in the form of pellets or paste. Plant extract is being eco friendly and very cost effective; the presented method can be economic and effective alternative therapy.

7. Dhandapani A et al.\cite{161} (2011) studied larvicidal and smoke repellent activity of aqueous extract of the plant *Cassia occidentalis* and developed a simple high performance thin layer chromatographic (HPTLC) method for the analysis of flavonoid in ethanol extracts of *Cassia Occidentalis*. Larvicidal potential of ethanol extract of *Cassia Occidentalis* was tested against the larvae of *Anopheles Stephensi*. The ethanol extract of *Cassia Occidentalis* were found most effective with LC50 value of 60.69%, 64.76%, 67.78%, 70.56%, 92.21% of I, II, III, IV and pupa respectively. The smoke toxicity was more effective against the *Anopheles stephensi*. In the present study also the *C.occidentalis* ethanol extract showed larvicidal activity against the malarial vector *A. stephensi* at a dose equivalent to LC50 ranging between 60.69 %, 64.76
% 67.78 %, 70.56 %, and 92.21%, for I, II, III, IV instar larvae and pupa Respectively, The result of this study indicate that C.occidentalis leaf extracts enhance the larvicidal and pupicidal activity, the leaves and pods enhances in the smoke repellency test it may be an effective alternative to conventional synthetic insecticides for the control of A. stephensi. The amount of flavonoid in the extracts has been estimated by comparing the peak area using the rutin as standard. Silica gel 60F254 TLC 3 x 10 plate was used as stationary phase and Ethyl acetate-butanone-formic acid-water in ratio of (5:3:1:1) was used as mobile phase. 1% ethanolic aluminum chloride reagent was used as derivatizing reagent and the spots developed was scanned at UV 366 nm. Development of plate showed different spots of flavanoids in the range of Rf values of between 0.11 to 0.97.

8. Daniya SY et al.\textsuperscript{[162]} (2011) carried out phytochemical screening, proximate analysis and mineral composition of Cassia occidentalis seed extract. The seeds of Cassia occidentalis were found to be rich in proteins, carbohydrates and minerals. They were found as a good source of antioxidant micronutrients like iron, calcium, potassium, sodium and magnesium. The plant was also studied for phytoconstituents presents. The results showed presence of carbohydrates, terpene, steroids, sugar and tannins. Alkaloids, saponins and glycolsides were found to be absent.

9. Kathirvel A et al.\textsuperscript{[163]} (2011) performed phytochemical screening of Cassia occidentalis Linn. in petroleum ether, chloroform and methanolic extracts. The chloroform and methanolic extracts of both flower and seed were found to contain flavonoids, alkaloids, phenolics, tannins, steroids, glycosides and anthraquinones. The antioxidant potential of flowers and seeds in different solvent extracts were evaluated by various biochemical assays namely, DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power activity. Their SC50 and EC50 values were determined to evaluate the therapeutic potential, in which seeds were found to have higher antioxidant activity revealed by
lower SC50 and EC50 value. The total phenol, flavanoid, flavonol and tannin content were determined for both parts to study the free radical scavenging property. The seeds were found to have higher antioxidant activity when compared to flowers in various solvent extracts indicating their pharmacological property.

10. **Mirtes GB et al.**\textsuperscript{[164]} (2011) performed a pre-clinical safety evaluation of hydroalcoholic extract of *Cassia occidentalis* stem and leaf in male and female Wistar rats. They performed acute toxicity acute toxicity tests, in which four groups of rats (n = 5/group/sex) were orally treated with doses of 0.625, 1.25, 2.5 and 5.0 gm/kg and general behavior, adverse effects and mortality were recorded for up to 14 days. In subacute toxicity assays, animals received *Cassia occidentalis* by gavages at the doses of 0.10, 0.50 or 2.5 gm/kg/day (n = 10/group/sex) for 30 days and biochemical, hematological and morphological parameters were determined. The study revealed that *Cassia occidentalis* did not produce any hazardous symptoms or death in the acute toxicity test, showing a LD50 higher than 5 gm/kg. Subacute treatment with *Cassia occidentalis* failed to change body weight gain, food and water consumption and hematological and biochemical profiles. In addition, no changes in macroscopical and microscopical aspect of organs were observed in the animals. The finding reveals the plant as safe to use for human.

11. **Ravikumar A et al.**\textsuperscript{[165]} (2011) studied efficacy of ethanolic extract from leaves of *Cassia occidentalis* against CCl\textsubscript{4} (Carbon tetrachloride) induced oxidative stress using Wistar albino rats. The antioxidant activity was assessed by monitoring the levels of lipid peroxides, antioxidant enzymes like glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase, and non-enzymic antioxidants like reduced glutathione, vitamin-C, vitamin-E, cereloplasmin and uric acid in the liver tissues. Administration of CCl\textsubscript{4} increased the level of lipid peroxides decreasing the activities of enzymatic and non-enzymatic antioxidants. Pre-treatment with ethanolic extract significantly prevented the alterations induced by CCl\textsubscript{4} and
maintained a near normal antioxidant status. Decreased activities of enzymes in CCl₄ intoxicated rats and their reversal in the ethanolic extract treated rats shows the potency of ethanolic extract in combating CCl₄ induced oxidative stress.

12. **Saidu AN et al.**[^166] (2011) investigated effect of aqueous extract of *Senna occidentalis* leaves on typhoid fever induced albino rats. Preliminary phytochemical screening of the plant extract of *Senna occidentalis* was also carried out which revealed presence of flavonoids, cardenolides, saponins, anthraquinones and alkaloids. Results of animal activity revealed that the aqueous extract of *Senna occidentalis* has the potential for typhoid fever treatment.

13. **Basha SI et al.**[^167] (2011) evaluated the anti-inflammatory effect of ethanolic extract of *Cassia occidentalis* seeds on acute inflammation (carrageenan-induced paw edema) and sub-acute inflammation (cotton pellet granuloma) in animal models. Animal used were Wistar albino rats (150-200 g) of either sex. The results revealed that ethanolic extract of *C. occidentalis* seeds at doses of 500 and 1000 mg/kg produced significant reduction in paw edema in acute inflammation model and reduced granuloma formation in sub-acute model of inflammation which indicates that the ethanolic extract of *C. occidentalis* seeds possesses anti-inflammatory effects in both acute and sub-acute inflammatory conditions.

14. **Yadav RN et al.**[^168] (2011) did isolation and structure elucidation of three new compounds from seeds of *Cassia occidentalis*. These compounds have been characterised as 5,7-dihydroxyflavone-5-O-β-D-xylopyranosyl- 7-O-α-L-rhamnopyranosyl - (1->3) - O - α - L - arabinopyranoside (1), 3,5,7,3’,4’-pentahydroxyflavone - 3 - O - α - L - rhamnopyranosyl - 7 - O - β - D - glucopyranosyl - (1->3) - O - β - D - xylopyranoside (2) and 5,7,3’, 4’-tetrahydroxy - methoxylavone - 5 -O - α - L-arabinopyranosyl - (1->4) - O - α - L - rhamnopyroxy - (1->3)-O - β - D - galactopyranoside (3). Respectively by various color reactions,
chemical degradations and spectral analysis. These compounds had been evaluated against various bacteria and fungi for anti microbial activity by filter paper disk diffusion method. Compound (1), (2) and (3) showed good result of antimicrobial and antifungal action but maximum antibacterial and antifungal activity was found with compound (3).

15. **Arya V et al.**\(^{[169]}\) (2010) tested different organic and aqueous extracts of leaves of *Cassia occidentalis* L (Caesalpiniaceae) for their antimicrobial activity against seven human pathogenic bacterial and two fungal strains by disk diffusion assay. Among these extracts, methanol and aqueous extracts showed significant antimicrobial activity against most of the tested microbes. The most susceptible microorganism was *P. aeruginosa* followed by *P. mirabilis* and *Candida albicans*.

16. **Egharevba et al.**\(^{[170]}\) (2010) studied antimicrobial effect of different extract i.e. aqueous methanol, hexane, ethyl acetate and methanol of *C. occidentalis*. Zone of Inhibition using different extracts were measured by Well Diffusion Method. The extracts were selectively activity against food pathogens like the *Staphylococcus aureus* and enteric organisms like *Streptococcus faecalis*, *Shigella dysenteriae* and *Bacillus* species. The methanolic and ethyl acetate extract showed some activity against Candida, Microsporum and Trichophyton which supports the traditional use of *Cassia occidentalis* for the treatment of various infectious diseases in different regions of the world.

17. **El-Kamali HH et al.**\(^{[171]}\) (2010) studied antibacterial activity of eight species of selected plantshaving a history of use in Sudanese traditional medicine for the treatment of infectious diseases. Phytochemical screening of these plants was performed for constituents like alkaloids, flavonoids, tannins, anthraquinones, saponins and volatile oils. Moisture, ash, crude fibres and soluble ethanol extractive contents had been carried out. The antibacterial screening of the ethanol extracts of the selected plants was performed by the agar well diffusion method against clinical isolates Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus*...
subtilis) and Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). All the eight ethanolic extracts showed good activity against four tested bacteria. Ethanolic extracts of C. occidentalis, C. tora, C. senna, R. minima var. memnonia, A. maritime and C. nervatus showed equal or nearly equal antibacterial activity both against Gram-positive and Gram-negative bacteria. Compared to the standard, C. occidentalis, C. nervatus, C. schoenanthus ssp. proximus ethanolic extracts exhibited a broader spectrum of antibacterial activity.

18. Chinnala KM et al.\textsuperscript{[172]} (2010) studied antimicrobial activity of petroleum ether (60-80ºC), chloroform, and methanol extracts of Cassia occidentalis roots against various strains of bacteria and fungi. Minimum Inhibitory Concentration (MIC) of the extracts were determined by broth dilution Method and the zone of inhibition of the extract was determined by agar disc diffusion method. The results revealed that the petroleum ether extract and chloroform extract have almost similar action on all types of micro-organisms but the methanol extract possess higher activity than other two and they are more effective towards gram-positive bacteria than gram-negative bacteria in a concentration dependent manner and were comparable with the standard drugs. (B. subtilis and least against Vibrio cholera). The preliminary phytochemical studies revealed the presence of lipids, proteins, tannins, saponins, flavonoids, triterpenoids, alkaloids in the extracts are likely to be responsible for the observed antimicrobial activity.

19. Bhagat M et al.\textsuperscript{[173]} (2010) evaluated In vitro citotoxicity and antibacterial properties of Cassia occidentalis plant via alcoholic, hydro alcoholic and aqueous extract against eight human cancer cell lines from six different tissues and four bacterial strains. Results revealed that aqueous extract showed maximum activity against six cell lines in does dependant manner. The plant can be used for molecular development as anti cancer and anti bacterial drug.
20. Janaky R et al.\textsuperscript{[174]} (2010) did comparative studies of secondary metabolites investigation used in pharmacognostic drug research using qualitative test on cassia occidentalis leaf and stem. The samples extracted using various solvents like ethanol, methanol, ethyl acetate and water to detect the presence of active components. The phytochemical screening revealed the presence of carbohydrate glycosides, alkaloids, phenols, flavanoid, aminoacid, coumarine and phytosterols. Since it contains high proportion of phenols and alkaloid, it is reliable to possess antioxidant and anticancer activity.

21. Kayembe JS et al.\textsuperscript{[175]} (2010) studied anti-malarial activity of 20 quinones isolated from Cassia alata, Cassia occidentalis, Garcinia kola and Ocimum basilicum was investigated In vitro using the micro dilution test of Desjardin by a visual evaluation on thin blood smears. The six quinones isolated from C. occidentalis, three from C. alata and three from O. basilicum were found to be the most active with an IC50 value of below 1 μg/ml. The others quinones showed a moderate activity with IC50 values of between 5 and 20 μg/ml.

22. Mehta S et al.\textsuperscript{[176]} (2010) studied In vitro antioxidant activity of methanol extract of Cassia occidentalis seeds using by DPPH free radical scavenging, FRPA, Lipid peroxidation by thiobarbituric acid assay methods. The analysis showed considerable reducing power of the extract under test, which increased in concentration dependent manner. Total phenolic content estimation was done using Folin-Ciocalteu reagent and was found to be 0.75% w/w. The present study revealed that the methanol extract of seeds have antioxidant potential and represent a potential source of medicine.

23. Saravanan S et al.\textsuperscript{[177]} (2010) studied anti inflammatory efficiency of chosen two plants Cassia occidentalis and Lannea coromandelica by delayed type hyper sensitivity method using mice. The inbred Swiss mice were divided into four groups, each groups have six animals. One group (Group - I) served as control and other groups (Group II - IV)
were received antigen along with cyclophosphamide (Standard immunosuppressive drug) and two plant extracts respectively. After treatment, the antigens were injected in the foot pad at 10% doses. The foot pad thicknesses were observed and this thickness is much reduced when the antigen was administered with cyclophosphamide (11.44 to 9.07 %). The plant extracts of *Lannea* and *Cassia* along with antigen showed no thickness or edema, only redness. Thus the present study confirmed the anti-inflammatory effect of the *Cassia occidentalis* and *Lannea coromandelica* studied.

24. **Rani SM et al.**[178] (2010) studied protective role of methanol fraction of leaves of *Cassia occidentalis* Linn and its pure compound chrysophanol against paracetamol-induced hepatotoxicity in rats. The findings reveled that Oral administration of chrysophanol and methanol fraction significantly normalized the values of SOD, CAT, GPx, GSH, Vitamine-C and Vitamine-E And the elevated serum enzymatic levels of AST, ALT, ACP and ALP were significantly restored towards normalization by pre-treatment with Chrysophanol and methanol fraction. The histopathological studies were also done which also confirmed the hepatoprotective nature of the extracts. The results of this study strongly indicate that *Cassia occidentalis* has potent hepatoprotective action against paracetamol induced hepatic damage in rats.

25. **Shreejith G et al.**[179] (2010) carried out anti allergic, anti inflammatory and anti lipidperoxidant activity of ethanolic extract of whole plant *Cassia occidentalis* using different *In vivo* and *In vitro* models like mast cell degranulation study, human RBC stabilization, carrageenan induced mouse paw oedema inhibition and reduction in malondialdehyde levels of murine hepatic microsomes.

26. **Sini KR et al.**[180] (2010) studied ethanol and water extracts of the leaves of the plant *Cassia occidentalis* Linn for their analgesic and antipyretic
effect using acetic acid induced writhing test, hot plate test and tail immersion test in mice and yeast induced pyrexia method in rats. The results of the statistical analysis showed that ethanol and water extracts of *Cassia occidentalis* had significant dose dependent analgesic and antipyretic effect.

27. **Wagh S et al.**[181] (2010) studied decoction of *Cassia occidentalis* seed for its antimicrobial activity against Gram-negative bacteria like *Escherichia coli* ATCC 10536, *Salmonella typhimurium* ATCC 23564, *Salmonella typhi* ATCC 531, *Klebsiella pneumoniae* MTCC 109, *Enterobacteraerogenes* ATCC 13048, *Proteus vulgaris* NCIM 2027 and *Pseudomonas aeruginosa* ATCC 27853. Gram-positive bacteria like *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 and fungi like *Aspergillus flavus* MTCC 277, *Candida albicans* MTCC 3017, *Candida tropicalis* MTCC 184, *Candida glabrata* MTCC 3019, *Trychophyton rubrum* MTCC 3272, *Microsporum gypseum* MTCC 4479 and *Epidermophyton floccosum* MTCC 613. Among the tested Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 was found most sensitive. The minimum inhibition concentration was found to be 6.25 mg/ml. The decoction of *Cassia occidentalis* has shown potential antibacterial activity against four Gram-negative bacteria including *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter aerogenes* and *Pseudomonasaeruginosa*. Among them *Pseudomonas aeruginosa* was found most sensitive. In case of Gram-positive bacteria tested, only *Staphylococcus aureus* was found sensitive. Among the fungi *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Trychophyton rubrum* and *Epidermophyton floccosum* were found sensitive except *Microsporum gypseum*. Among them all *Aspergillus flavus* have shown highest sensitivity towards the decoction. The results indicate that *Cassia occidentalis* has broad spectrum antimicrobial potential against antibiotic resistant microorganisms.
28. **Yadav JP et al.**[^182] (2010) reviewed *Cassia occidentalis* plant for its different activities like antibacterial, antifungal, antidiabetic, anti-inflammatory, anticancerous, antimutagenic and hepatoprotective activity. The plant is also reviewed for its constituents like achrosin, aloe- emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol etc. from which some have been isolated. The study summarized summarizes the information concerning the botany, ethnopharmacology, phytochemistry, biological activity and toxicity of the *C. occidentalis* plant.

29. **Sheeba M et al.**[^183] (2009) studied wound healing property of crude extract of *Cassia occidentalis* L leaves and a pure compound chrysophanol isolated from it was evaluated in excision and dead space wound models. Chrysophanol was found to possess significant wound healing property than methanol crude extract. Study concluded *C. occidentalis* as a good source of wound healing compound.

30. **Vashishtha VM et al.**[^184] (2009) reviewed clinical and pathological feature of acute toxicity due to *Cassia occidentalis*. Several animal studies have documented that fresh or dried beans are toxic. Ingestion of large amounts by grazing animals has caused serious illness and death. The toxic effects in large animals, rodents and chicken are on skeletal muscles, liver, kidney and heart. The predominant systems involved depend upon the animal species and the dose of the beans consumed. Brain functions are often affected. Gross lesions at necropsy consist of necrosis of skeletal muscle fibres and hepatic centrilobular necrosis. Renal tubular necrosis is less frequent. Muscle and liver cell necrosis is reflected in biochemical abnormalities. The median lethal dose (LD 50) is 1 g/kg for mice and rats. Toxicity is attributed to various anthraquinones and their derivatives and alkaloids, but the specific toxins have not been identified. Data on human toxicity are extremely scarce. This review summarizes information available on *Cassia* toxicity in animals and compares it with toxic features reported in children.
clinical spectrum and histopathology of \textit{C. occidentalis} poisoning in children resemble those of animal toxicity, affecting mainly hepatic, skeletal muscle and brain tissues. The case-fatality rate in acute severe poisoning is 75-80 per cent in children.

31. 	extbf{Nuhu AA et al.\cite{185} (2008)} studied hypoproteinaemic effect of aqueous extract of fresh leaves of \textit{Cassia occidentalis} on albino rats. Phytochemical screening revealed presence of tannins, anthraquinones, saponins, cardenolides, flavonoids, alkaloids. Aqueous extract of this herb was studied for its effect on serum total proteins, albumin, bilirubin, alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP), and hepatoprotective potential of the plant extract was also evaluated. The results revealed that the studied extract is useful to treat hepaticities with slight toxicity.

32. 	extbf{Adamu HM et al.\cite{186} (2006)} studied antifungal effect of methanolic extracts of \textit{Cassia occidentalis} Linn. and other plants of this family by agar diffusion method. The activity was studied against dermatophytes like \textit{Trichophyton rubrum}, \textit{T. mentagaphytes}, \textit{microsporum caris} and \textit{Aspergillus fumigates}. The findings showed that all the test fungi were inhibited significantly by all the extracts as compared to the water control.

33. 	extbf{Saganuwan A et al.\cite{187} (2006)} evaluated \textit{In vitro} antimicrobial activity and phytochemical constituents of \textit{Cassia occidentals}. \textit{Cassia} leaves were extracted using methanol, hexane, chloroform and water. Serial concentrations: 50 60, 70, 80, 90 and 100 \% methanol, hexane, chloroform and aqueous extracts were prepared and sterilized. The bacterial isolates used were \textit{E. coli}, \textit{P. multocida}, \textit{S. typhi}, \textit{S. typhimurium}, \textit{S. pyogenes}, \textit{S. pneumoniae} and \textit{K. pneumonia} and were authenticated using biochemical and serological methods. The disc agar diffusion method was performed on 70 Mueller-Hinton agar plates, 10 per microorganism, using serial diffusion concentraton 500, 600, 700, 800, 900 and 1000 mg of hexane, methanol, chloroform and water. The
results showed that all the extracts of Cassia occidentalis have antimicrobial activity on *E coli* at concentrations between 900 – 1000 mg. *E. coli* was most susceptible to hexane extract at concentration ranges between 500 – 1000 mg, there was no antimicrobial activity exhibited against the other tested microorganisms. Phytochemical analyses showed the presence of alkaloid, tannin, saponin, glycoside and flavonoid. Steroid was absent.

34. **Tona L et al.**[188] (2004) studied the *In vitro* antiplasmodial activity of seven ethanolic extracts and twenty fractions from the partition of the initial ethanolic extracts from seven African medicinal plants used in the Democratic Republic of Congo (DR Congo) for the treatment of malaria. The most active ethanolic extracts found were those from *Cassia occidentalis* leaves, *Euphorbia hirta* whole plant, *Garcinia kola* stem bark and *Phyllanthus niruri* whole plant. Their respective petroleum ether soluble fractions also exhibited an antiplasmodial activity. Ethanolic extracts from *Vernonia amygdalina* leaves, *Tetracera poggei* leaves and *Morinda morindoides* leaves were less active, but their petroleum ether fractions exhibited a pronounced antiplasmodial activity. The same observation could also be made for the petroleum ether fraction from *Cassia occidentalis*, *Euphorbia hirta*, *Garcinia kola* and *Phyllanthus niruri*. Iso amyl alcohol fractions from *Euphorbia hirta*, *Phyllanthus niruri* and *Vernonia amygdalina* showed IC50 values less than 3 μg/ml, and from *Cassia occidentalis*, *Garcinia kola*, *Morinda morindoides* and *Tetracera poggei* between 10 and 50 μg/ml. The observed antiplasmodial activity was supposed to be due to presence of terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinones.

35. **Ajagbonna OP et al.**[189] (2001) studied the relaxant effects of an aqueous extract of the leaf of plant *Cassia occidentalis* in rat aortic rings with or without intact endothelium. The extract inhibited contraction elicited by noradrenaline and Potassium Chloride dose dependently. It also relaxed aortic rings precontracted with 10-7 M
noradrenaline and 50m M Potassium Chloride. This relaxation did not require the presence of an intact vascular endothelium and was not affected by indomethacin (Prostacylin inhibitor) and methylene blue.

2.2 Review of Activity of Drug on Animals

2.2.1 Clonidine induced peritoneal mast cell degranulation

1. Janadri S et al.[190] (2011) studied the effect of nanosizing on Bio-efficacy of Shwaskuthar rasa- A herbomineral formulation for asthma and allergy. The antiasthma and antiallergic activity of nanosized Shwaskuthar Rasa was evaluated in albino rats by clonidine induced mast cell degranulation and catalepsy. For standard treatment, disodium chromoglycate 50 mg/kg, i.p. was administered. There was significant percentage protection of mast cell (44.13%) found in the Shwaskuthar rasa treated group compared to control group (27.16%) and significant inhibition in the catalepsy over the control group. Size reduction shows that Shwaskuthar Rasa was significant protection on mast cell degranulation and catalepsy in rats which is fineness dependant i.e. smaller the particle size, better the antiasthmatic and antiallergic effect. Shwaskuthar rasa showed the least inhibition in the catalepsy. So, they concluded that efficacy of the Shwaskuthar Rasa increases as the particle size decreases facilitating the drug to cross the biological barriers thus increasing the bioavailability of the formulation which may eventually be responsible for its enhanced antihistaminic activity.

2. Mali PR et al.[191] (2011) studied antiasthmatic activity of aqueous extract of roots of Mimosa pudica Linn. They were produced asthma in mice by clonidine induced mast cell degradation. The mean percentages of mast cells were determined by counting 100 cells from each subcutaneous spread. The aqueous extract of Mimosa pudica showed significant reduction in mast cell degradation. The prevention
of mast cell degradation indicates a possible stabilizing effect on the biomembrane of mast cells.

3. **Kumar D et al.** [192] (2010) studied antiasthmatic and antiallergic potential of methanolic extract of leaves of *Ailanthus excelsa* Roxb., Simaroubaceae. Methanolic extract of leaves of *A. Excels* was evaluated using *in vitro* goat tracheal chain preparation model and *in vivo*- Milk induced leucocytosis, eosinophilia, Clonidine induced catalepsy in mice model while passive paw anaphylaxis and Clonidine induced mast cell degranulation in rat model. The extract also showed the presence of quercetin which is flavonoid and detected on the preparative TLC plate with the help of standard quercetin. Dose response studies of methanolic extract of leaves of *A. excelsa* Roxb. were conducted at 100 μg/ml *in vitro* and 100, 200, 400 mg/kg *p.o. in vivo* models. The treatment with methanolic extract of *A. excelsa* at different dose level showed the significant antiasthmatic activity. Inhibition or decrease the release of inflammatory mediators potentiates the antiasthmatic as well as antiallergic activity of methanolic extract of leaves of *A. excelsa*.

### 2.2.2 Histamine Induced broncho spasm in guinea pig

1. **Misra KH et al.** [193] (2014) evaluated antiasthmatic effect of ethanol extract of *Piper betel* Linn. against histamine induced bronchospasm in guinea pigs. Asthma was induced by 0.2% histamine aerosol. For standard treatment, Chlorpheniramine was administered as antihistaminic agent. An ethanolic extract of *Piper betel* Linn. at a dose of 100 mg/kg body weight and 200 mg/kg body weight was studied in guinea pigs for its effect in asthma. Preconvulsive time (PCT) was noted for all groups. They concluded that the ethanolic extract of *Piper betel* Linn. has significantly prolonged the latent period of convulsions (PCT) as compared to control following the exposure of histamine aerosol. A study showed protection against histamine induced
experimental bronchial asthma in guinea pigs which may be due to anti-inflammatory, antioxidant and antihistaminic activity.

2. **Sehgal R et al.** [194] (2013) studied the effect of various plant extracts on histamine aerosol induced bronchospasm in guinea pigs. Bronchospasm was induced in guinea pigs by exposing them to 1% histamine aerosol under constant pressure in an aerosol chamber (24 X 14 X 24 cm) made of perplex glass. Animals were exposed to 1% histamine aerosol under a histamine chamber. The end point of preconvulsive dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from the chamber and exposed to fresh air. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders, causes bronchospasm which cause precipitation of bronchial asthma.

3. **Kumar A et al.** [195] (2002) studied effect of methanolic extract of *Benincasa hispida* against histamine and acetylcholine induced bronchospasm in guinea pigs. Before drug treatment, the animals were placed in the histamine chamber and exposed to aerosol of histamine acid phosphate (0.25%) under a constant pressure of 40 mm Hg from the inbuilt nebuliser. The preconvulsive time (PCT) was determined from the time of exposure to the onset of dyspnea leading to the appearance of convulsions which is known as preconvulsive dyspnoea (PCD). The protective effect against bronchospasm induced by histamine aerosol may be mediated by antihistaminic activity (H1 receptor antagonism).

### 2.2.3 Carragenan induced rat paw edema

1. **Amdekar S et al.** [196] (2012) studied anti-Inflammatory activity of lactobacillus on carrageenan-induced paw edema in male Wistar rats. Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay. Edema was induced by subplantar
injection of 100μL of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws. Treatment was administered before 30 minutes prior to carrageenan injection. Paw thickness were measured just before the carrageenan injection, that is, at “0 hour” and then at 1, 2, 3, 4, and 24th hour after carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at “0 hour” and paw thickness at respective hours. Lactobacillus showed significant decrease in the paw thickness of rat.

2. **Xu Z et al.** [197] (2012) studied anti-inflammation effects of hydrogen saline in LPS activated macrophages and carrageenan induced paw oedema. For the induction of inflammation, 0.5 ml phlogestic agent (Carrageenan, 1%) was administered by subcutaneous injection into the right hind paw. The paw volume was measured by a volume measuring instrument, Plethysmograph. Hydrogen saline showed dose-dependent inhibitory activity in carrageenan-induced paw oedema.

3. **Paschapur MS et al.** [198] (2009) studied effect of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* for its anti-inflammatory activity in rodents. The anti-inflammatory activity was evaluated using acute inflammatory models like; carrageenan-induced paw oedema and chronic models like; cotton-pellet induced granuloma and carrageenan-induced air-pouch model in rats. The biochemical parameters like serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), lipid per oxidation and alkaline phosphatase (ALP) were also estimated as supportive studies. Oral administration of the extract at the doses 150 and 300 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan- induced hind paw oedema, p < 0.0001) and chronic (cotton pellet granuloma and carrageenan-induced air-pouch
models, p < 0.0001) of inflammation. The extract also showed significant (p < 0.0001) results for biochemical parameters.

2.2.4 Neutrophile adhesion test

1. Agrawal SS and Talele GS\textsuperscript{[199]} (2011) studied bioactivity guided isolation and characterization of the phytoconstituents from the \textit{Tridax procumbens}. After 14 days of treatment, blood samples were collected by retroorbital puncture in heparinized vials and subjected to total as well as differential leukocyte count. After initial counts the blood samples were incubated with 80 mg/ml of nylon fibers at 37 °C for 15 min. The incubated samples were again analyzed for total and differential leukocyte count. The product of total leukocyte count and % neutrophil known as neutrophil index was determined for each of the respective groups. They determined % neutrophil adhesion.

2. Chakraborthy GS\textsuperscript{[200]} (2009) studied the immunomodulatory activity of \textit{Aesculus indica}. \textit{Aesculus indica} was administered orally at doses of 50 and 100 mg/kg to healthy rats. The assessment of immunomodulatory activity was carried out by testing the humoral (antibody titre) and cellular (foot pad swelling) immune responses to the antigenic challenge by sheep RBCs and by neutrophil adhesion test. On oral administration of the extract, a significant increase in neutrophil adhesion and delayed type hypersensitivity response whereas the humoral response to sheep RBCs was unaffected. Thus \textit{Aesculus indica} significantly potentiated the cellular immunity by facilitating the foot pad thickness responses to the sheep RBCs in sensitized rats. The responses were statistically significant when they were compared with the control. They concluded that \textit{Aesculus indica} shows a significant stimulation of the cell mediated immunity and no effects on the humoral immunity.

3. Fulzele SV et al.\textsuperscript{[201]} (2002) studied immunostimulant activity of \textit{Ashtamangal ghrita} in rats. \textit{Ashtamangal ghrita} was administered
orally at doses of 150 mg/kg/day and 300 mg/kg/day to healthy rats. The assessment of immunostimulant activity was carried out by testing the humoral (antibody titre) and cellular (foot pad swelling) immune responses to the antigenic challenges with sheep RBCs and by neutrophil adhesion test. Orally administered *Ashtamangal ghrita* showed a significant increase of test parameters viz. neutrophil adhesion, haemagglutinating antibody titre (HAT) and delayed type hypersensitivity (DTH) response. In rats immunized with sheep RBC, AG enhanced the humoral antibody response to the antigen and significantly potentiated the cellular immunity by facilitating the footpad thickness response to sheep RBC in sensitized rats. With a dose of 300 mg/kg/day the values of HAT and DTH responses were 455.08 ± 0.75 and 31.0 ± 10.72 respectively, in comparison to the control group. These differences were statistically significant. They concluded that the immunostimulatory activity of *Ashtamangal ghrita* *in vivo* in rats.

### 2.3 Review of formulation

1. **Kagalkar AA et al.** (2014) developed and evaluated herbal fast dissolving tablet from *Tectona grandis* Linn. In this study, MCC is used as diluent and sodium saccharin as sweetening agent for the formulation of tablets. Attempts were made to enhance dissolution rate along with faster disintegration using superdisintegrants like Crospovidone, Sodium starch glycolate (SSG) and mixture of crospovidone and sodium starch glycolate in the formulation of tablets. The tablets were subjected to weight variation, drug content uniformity, hardness, friability, wetting time, *In vitro* dispersion time, *In vitro* drug release studies and *In vivo* studies. *In vivo* studies showed that formulation F1 has antidiabetic activity. The comparative study of several superdisintegrants yielded a conclusion that Crospovidone at 3% concentration are suitable for the preparation of Herbal fast dissolving tablets which will satisfy all the criteria and official limits.
2. Monton C et al.\textsuperscript{[203]} (2014) developed a fast disintegrating tablet formulation of Wat Pho hypnotic formula, a classical Thai traditional hypnotic drug. In addition, a preformulation study and physical properties of the finished products were investigated to select the best formulation for further study. Tablets of the six formulations (D1-D6) were prepared by the direct compression method. The preformulation studies were investigated including angle of repose, bulk density, tapped density, compressibility index, and Hausner ratio. The suitable formulas were compressed into tablets and then were evaluated for physical properties following the United State Pharmacopeial method including weight variation, friability, thickness, hardness, and disintegration time. The results show that D3 formula contained 500 mg of herbal mixture, 137 mg of avicel PH 102, 6.5 mg of colloidal silicon dioxide, and 6.5 mg of magnesium stearate per tablet. It was the best formulation as it showed good flowability (angle of repose was 35°, compressibility index was 21%, and Hausner ratio was 1.26); weight variation was narrowed and friability was less than 1%, hardness was 5.69 kiloponds, and the tablets completely disintegrated in 1.31 minutes. The finished products showed less weight variation, less friability due to appropriate hardness and thickness. The tablet completely disintegrated within a few minute. Further studies of this formulation are necessary to evaluate other physical properties and chemical properties of the formulations.

3. Kulkarni U et al.\textsuperscript{[204]} (2011) developed a formulation of fast disintegrating tablets containing Fenugreek seed powder by wet granulation technique. Crospovidone and Plateau ovata powder were used as superdisintegrants and also by taking the advantage of self-disintegration property of fenugreek seeds. Preformulation studies indicated that the powder blend was not having free flowing nature. So wet granulation technique was adopted and appropriate tablet formulations were developed. Formulations were optimized to develop tablets having minimum possible disintegration time. Tablets were evaluated for hardness, weight variation, friability, wetting time,
disintegration time and stability. The results of hardness of the tablets were in the range of 5.15-6.25 kg/cm². Friability was less than 1% in all the formulations, indicated that tablets had a good mechanical resistance. The results of *In vitro* disintegration time study revealed that the formulations containing both super disintegrants crospovidone and plantego ovata (F7) were showed disintegration time of 14.52 seconds. It may be due to wicking action of crospovidone and swelling nature of the plantego ovata and fenugreek powder. In wetting time study, the wetting time was decreased with increased concentration of superdisintegrants. All these results revealed that fast disintegrating tablets of fenugreek seeds powders could be prepared at any level of superdisintegrants. Stability study was also conducted as per ICH guidelines and all formulations were found to be stable. The results concluded that fast disintegrating tablets of fenugreek seeds powders will leads to improved effectiveness and hence better patient compliance.

4. **Salunkhe VR et al.**[205] (2009) developed a formulation and do real time stability studies of herbal oral liquids containing natural sweetener. They develop herbal oral liquids containing *Withania somnifera, Asparagus racemosus, Ipomea purga, Glycyrrhiza glabra, Terminalia chebula, Curcuma zedoria, Tinospora cordifolia, Cyperus rotundus, Tribulus terrestris, and Sida cardifolia* as active ingredients and *Stevia rebaudiana* as natural sweetener. Standardization was carried out using applicable parameters like color, odor, general appearance, taste, pH, viscosity, surface tension, clarity, specific gravity and other additional parameters like microbial count, TLC profile, HPTLC fingerprint, determination of heavy toxic metal ions and pesticide residue. Sweetness potency was determined by taste evaluation method. Antioxidant potential of bitter and sweet herbal oral liquid was studied in terms of percentage scavenging by oral liquid and maximum inhibitory concentration i.e. IC50 values also determined. The sample showed better activity in quenching nitric oxide radicals with a IC50 value 9.64, 10.01 μg/ml and DPPH (1, 1-
diphenyl -2-pieryl hydrazyl) radicals with an IC50 value of 11.12, 11.42 μg/ml. However the extract also showed encouraging responses in generating superoxide with IC50 value of 12.95, 12.41 μg/ml. The activity was moderate in remaining antioxidant models. The results showed that sweet herbal oral liquid has a potent scavenging activity with increasing percentage inhibition. Heavy toxic metal ions (As, Pb, Hg, and Cd) and pesticide residues (Chloride & Phosphates) were totally absent. Use of this natural sweetener is most convenient, acceptable and palatable in sweet formulations.

2.4 Review of Phytochemistry and HPTLC

1. Sushma GS et al.[206] (2013) established the fingerprint profile of *Ficus nervosa* using high performance thin layer chromatography (HPTLC) technique. Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. The results of preliminary phytochemical studies confirmed the presence of alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenol. HPTLC finger printing of chloroform extract of leaf revealed 11 peaks with Rf values in the range of 0.07 to 1; ethyl acetate extract of leaf showed 11 peaks with Rf values in the range of 0.07 to 0.99 and 90% ethanolic extract of leaf revealed 13 peaks with Rf values in the range of 0.03 to 1. It can be concluded that HPTLC fingerprint analysis of leaf of *Ficus nervosa* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

2. Syed MH et al.[207] (2013) established the fingerprint profile of *Pisonea aculeata* using high performance thin layer chromatography (HPTLC) technique. Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system
equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. Results: Preliminary phytochemical screening of the extract showed the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids. HPTLC finger printing of chloroform extract of leaf revealed 14 peaks with $R_f$ values in the range of 0.03 to 0.95; ethyl acetate extract of leaf showed 6 peaks with $R_f$ values in the range of 0.04 to 0.94 and 90% ethanolic extract of leaf revealed 11 peaks with $R_f$ values in the range of 0.03 to 0.93. Conclusions: It can be concluded that HPTLC fingerprint analysis of leaf extract of *Pisonea aculeata* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

3. *Seasotiya L et al.*[208] (2014) did phytochemical screening and developed high performance thin layer chromatography (HPTLC) finger print profile of methanol and ethyl acetate extracts of leaves of *Cassia fistula*. Chromatographic technique was used for separation of components from different extracts of leaves. This study was planned to develop a HPTLC fingerprint profile of extracts in different solvents such as petroleum ether, toluene, ethyl acetate, chloroform, acetone and formic acid. A HPTLC method for the separation of the active constituents in extracts has been developed and TLC of these extracts on silica gel pre-coated aluminum plates of Merck by automatic TLC applicator and using solvent system toluene: ethyl acetate: formic acid::5:4:1 was performed. HPTLC profiling of the extract confirm about the presence of various phytochemicals. HPTLC finger print scanned at 400 nm for methanol and ethyl acetate leaf extracts revealed 15 and 16 peaks with $R_f$ values in the range of 0.06 to 0.99 and 0.02 to 0.98 respectively. The bands revealed presence of greenish, purple, pink and light yellowish orange bands showing the presence of steroids, terpenoids and saponins after spraying with anisaldehyde sulphuric acid reagent. The Phytochemical tests on methanol and ethyl acetate extracts of *C.
*fistula* leaves showed the presence of various phytoconstituents like alkaloids, saponins, flavonoids, phenols and tannins The HPTLC method for routine quality control of present species can be carried out using this method for extracts of plant and serve in qualitative, quantitative and was appropriate for standardization of the extract.

4. Kamboj A et al. [209] (2011) developed the high performance thin layer chromatography (HPTLC) finger print profile of various extracts of dried aerial parts (leaves, stem, and flower) of *Ageratum conyzoides*. Chromatographic techniques were used for separation of components from different extracts of plant parts. This study was planned to develop a HPTLC fingerprint profile of drug extracts from aerial parts of *Ageratum conyzoides* in different solvents such as petroleum ether, benzene, chloroform, acetone and ethanol (95%). A HPTLC method for the separation of the active constituents in *Ageratum conyzoides* extracts has been developed and TLC of these extracts on silica gel precoated aluminum plates of Merck by automatic TLC applicator and using solvent system chloroform: ethanol (9.8:0.2) was performed. In the present study, HPTLC finger print of various extracts of dried aerial parts of *Ageratum conyzoides* have been carried out and the results provide referential information for standardization. The HPTLC method for routine quality control of present species can be carried out using this method for different extracts of plant parts and serve in qualitative, quantitative and was appropriate for standardization of the drug. The HPTLC fingerprint is also suitable for rapid and simple authentication and comparision of differences among samples of identical plant resource.