Plants that have medicinal properties with active ingredients in some form or other are regarded as medicinal plants. Various medicinal plants have been used for years in daily life to treat diseases all over the world. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare. *Seabania grandiflora* (Fabaceae), commonly known as agati is a widely available, fast growing plant, generally popular for its animal fodder use. Traditionally the plant has been used for the treatment of head ache, used in fever as a tonic, in catarrh, as an astringent etc. It is claimed to have hypolipemic, anti ulcer and anti-inflammatory properties as well.

The main objectives of the present study are to extract the phytochemicals from *Sesbania grandiflora* leaves using solvents viz., petroleum ether, chloroform, methanol, ethanol and acetone, to evaluate the pharmacological properties of the extracts, to estimate the phytochemicals present in all extracts, to separate the chemical compounds present in biologically active extract, to elucidate the structure of the separated phytochemicals using advanced techniques and to evaluate the biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer activities and antigenotoxic effect.

*Sesbania grandiflora* leaves were collected from maruthuval malai in kanyakumari district (Tamilnadu, India) and identified by a plant taxonomist. The dried leaves were extracted in soxhlet apparatus using solvents like petroleum ether, chloroform, methanol, ethanol and acetone. The yield obtained from different solvents varied between 13 and 25%. The maximum yield was obtained with methanol where as
the minimum was with acetone. Qualitative assays were done for alkaloids, flavonoids, glycosides, tannins, anthraquinones, steroids and terpenoids. Among different extracts tested, methanol extract showed higher concentrations of the important phytochemicals (i.e) total alkaloids at the level of 1.9 mg/g dw, total phenols at a concentration of 28.4 mg GAE/g dw and total flavonoids at 5.5 mg QE/g dw where as ascorbic acid was found to be 119 mg/g dw. As the methanol extract was found to be rich in important phytochemicals, further pharmacological evaluations were done using this extract only. In the crude extract antibacterial, antifungal and antioxidant activities were studied. Presence of nutritive components like protein and carbohydrate were done also qualitatively. The proximate analysis of dried leaves showed 3.8 mg/g dw of protein, 5.2 mg/g dw of carbohydrate and 1.0mg/g dw of lipids. Free amino acids were found at a concentration of 3.4 mg/g dw.

From leaf extracts the content of minerals were analyzed using AAS which revealed the presence of calcium, phosphorus, potassium, copper and ferrous ions. Their respective concentrations were 0.15, 0.052, 0.1, 0.35 and 0.04 mg/ g dw.

Pigments like Chlorophyll a, Chlorophyll b and carotenoids were estimated spectrophotometrically and were found to be 2.8, 2.5 and 1.2 mg/g wet wt. Total protein in leaf sample was estimated following Lowry et al., 1951 method where as total lipid by Agarwal et al., 1987 method. Determination of total carbohydrates was done adopting Anthrone method. Total free amino acids were estimated by Ninhydrin method. Total alkaloid content was estimated by Sairam and Khanna (1971), where as total polyphenolics and total flavonoids were determined by Kale et al., 2010 method. Ascorbic acid content was done by the procedure given by Sadasivam and Balasubramanian (1987). Minerals (Ca, P, K, Cu and Fe) were determined using the method of AOAC (1990) using atomic absorption Spectrophotometer.
To evaluate the pharmacological potential of leaf extracts, antibacterial, antifungal activity were done using well assay. Minimum inhibitory concentration was determined using standard procedure.

Among the 10 bacterial pathogens tested, the methanol extract of *S. grandiflora* leaves showed highest activity against *S. aureus* (25mm), followed by *B. cereus* (18mm), *E. coli* (16mm), *P. aeruginosa* (11mm), *L. monocytogenes* (9mm), *S. typhi* (5mm), *V. cholerae* (5mm) and *S. flexneri* (5mm). It did not show any activity against *S. paratyphi* and *V. parahaemolyticus*. The MIC values obtained in the present study against various pathogens were in the range of 11 to 22 mg/mL.

The fungal pathogens *C. albicans*, a human pathogen and 10 different plant pathogens like *C. musae, S. roysii, A. niger, C. capsici, F. oxysporum, F. udum, B. cinera, A. alternate, R. solani* and *M. phaseolina* were tested, with crude methanol extract of *S. grandiflora*. The MIC value observed was 25mg/mL for *C. albicans*. However no activity was observed against the plant pathogens.

The crude methanolic extract was tested for various antioxidant assays to evaluate its potential. In the present investigation maximum total antioxidant activity of 4.2mg/mL was observed at crude methanolic leaf extract concentration of 0.32mg/mL, DPPH and free radical scavenging activities were checked at the concentration range of 100-500µg/mL. Highest activity was observed at higher concentration of crude (500µg/mL). DPPH, super oxide, hydroxyl and hydrogen peroxide radical scavenging activity percentage of crude methanolic extract was 87%, 81%, 89% and 82% respectively.

Isolation chemical components of methanol extract was done using Sephadex LH 20 column and preparative TLC was used to check individual compounds. Seven chemical components are present and were separated, but three components have
remarkable biological activities which were characterized by FTIR and NMR (\(^1\)H, \(^13\)C and 2D NMR). The isolated compounds were identified as lupeol (C\(_{30}\)H\(_{50}\)O), kemferol (C\(_{15}\)H\(_{10}\)O\(_{6}\)) and gallic acid (C\(_{7}\)H\(_{6}\)O\(_{5}\)) and their structures were elucidated based on the spectral data. The identified compounds were further evaluated for their pharmacological potential, based on their antimicrobial, antioxidant, anti-inflammatory, anticancer properties and antigenotoxic effect.

When 25\(\mu\)g/mL of lupeol was tested, it resulted in highest inhibitory activity to \(S.\) aureus with 45mm zone of clearance followed by \(B.\) cereus (40mm), \(E.\) coli (35mm), \(P.\) aeruginosa (30mm), \(L.\) monocytogenes (15mm) and \(V.\) parahaemolyticus. When crude extract showed lower activity to \(S.\) typhi and \(V.\) cholerae, lupeol did not show any inhibitory activity to these organisms. Surprisingly, when crude extract did not show any activity towards \(S.\) typhi and \(V.\) parahaemolyticus, lupeol showed lower inhibitory activity against these organisms (8\(\mu\)g/mL and 9\(\mu\)g/mL respectively). Gallic acid also showed most inhibitory activity against \(S.\) aureus (43mm), followed by \(B.\) cereus (32mm), \(E.\) coli (21mm) \(P.\) aeruginosa (27mm) and \(L.\) monocytogenes (15mm) whereas \(S.\) typhi and \(V.\) cholerae showed only 5mm zone of clearance. MIC for \(S.\) aureus was 2.8\(\mu\)g/mL which was found to be the least value and 11\(\mu\)g/mL was the highest value recorded for \(V.\) cholerae and \(V.\) parahaemolyticus. Kempferol also showed higher inhibitory activity compared to crude extract. This compound also most inhibited \(S.\) aureus (43mm), followed by \(B.\) cereus (25mm), \(E.\) coli (19mm) and \(L.\) monocytogenes (10mm) and \(S.\) typhi (5mm). Kempferol did not show inhibitory activity against \(P.\) aeruginosa, \(S.\) typhi, \(V.\) cholerae, \(S.\) flexeneri and \(V.\) parahaemolyticus. The MIC value against \(S.\) aureus was 2\(\mu\)g/mL where as it was 10\(\mu\)g/mL for \(L.\) monocytogenes. Among 10 pathogens tested, only 5 were inhibited. \(P.\) aeruginosa which was inhibited
by lupeol and gallic acid was resistant to kempferol. Moreover when crude extract could not inhibit *V. parahaemolyticus*, kempferol showed moderate activity to that pathogen.

In the antifungal activity, lupeol showed lower activity (7mm) towards *C. albicans*, a human pathogen. However when tested against plant pathogens like *C. musae, S. roysii, A. niger, C. capsici, F. oxysporum, F. udum, B. cinera, A. alternate, R. solani* and *M. phaseolina*, lupeol inhibited 6 pathogens with highest activity towards *B. cinera* (35mm), followed by *F. udum* (30mm) *C. musae* (30mm), *A. niger* (17mm) and *A. alternata* (10mm). Their respective MIC values were 25, 20, 50, 75 and 150 µg/mL. For the rest of the pathogens MIC values were above 200 µg/mL. In the antifungal activity, gallic acid inhibited *C. albicans* only at a moderate level (i.e.) 8mm. Regarding plant pathogens *F. udum* was most inhibited (18mm) by this compound followed by *A. alternata* (17mm), *B. cinera* (16mm), *A. niger* (15mm) and *C. musae* (15mm). However it did not inhibited other five fungi tested. Gallic acid showed a MIC value of 50 µg/mL against *F. udum, B. cinera* and *A. alternata* and a value of 75 µg/mL against *C. musae* and *A. niger*. For the rest of the fungi MIC values were > 150 µg/mL. Kemferol inhibited *C. albicans* at moderate level only. However compared to other compounds, the zone of inhibition observed was larger (13mm). Among the fungi tested *B. cinera* was the most inhibited (26mm), followed by *C. musae* (18mm), *A. niger* (10mm), *F. udum* (10mm) and *A. alternata* (10mm). The other fungi were found to be resistant to these compounds. Regarding MIC values, it was 50 µg/mL for *B. cinera*, 75 µg/mL for *C. musae* and 100 µg/mL for *A. niger*. For the rest of the pathogens MIC value was above 150 µg/mL.

Antioxidative potential of the compounds was extensively studied using various procedures viz., total antioxidant activity, DPPH assay, superoxide anion scavenging activity, hydroxyl radical scavenging assay, hydrogen peroxide scavenging activity as
per the methods given respectively by Pourmorad et al., 2006, Zhao et al., 2006, Liu et al., 1997, Kunchandy and Rao (1990) and Zhang (2000). Enzymatic antioxidants like superoxide dismutase, catalase, peroxidase, ascorbic acid oxidase were done by the methods described by Beauchamp and Fedovich (1976), Chance and Maehly (1995), Malik and Singh (1980) and Oberbacher and Vines (1965).

The isolated components were tested for antioxidant studies using DPPH assay. When lupeol was tested at the concentration range of 2-10µg/mL, respectively 14, 20, 48, 82 and 99% of scavenging activity was observed at 2, 4, 6, 8 and 10 µg/mL. Among the three isolated compounds, lupeol showed highest reducing activity. In the DPPH assay antioxidant activity, when the concentration tested were 2, 4, 6, 8 and 10 µg/mL, the scavenging activity observed were respectively 12, 15, 40, 76 and 92%. Among 3 compounds gallic acid showed the least reducing activity (92 %).

Anti inflammatory potential of lupeol, kempferol and gallic acid was done using Hamster chicks as animal model. Oral tumors were developed in the buccal pouch of hamsters by painting with 0.5% DMBA, a potent carcinogen. Total RNA from the hamsters tissues were extracted in control and hamsters treated with lupeol, kempferol and gallic acid. Isolated total RNA was reverse – transcribed to cDNA and RT-PCR was performed using a thermal cycler, to find the expression of NFkB gene using specific primers.the NFkB mRNA expression and fold increase pattern of control and experimental animals were compared. Among the three components, lupeol was found to be the most potent compound inhibiting the inflammatory response followed by kempferol and gallic acid.

The isolated components were performed anti proliferative assay using HEPG2 (Human Hepatoma Cancer Cell Lines) and MIC assay by which cell viability was calculated. When purified lupeol was used against HEPG2 cancer cell lines, it inhibited
the cell viability to 12.5% at a concentration of 1000 µg/mL and the IC₅₀ value was found to 62.5 µg/mL. In the anticancer/antiproliferative activity, Gallic acid inhibited HEPG₂ cell viability to 6.32% at a concentration of 1000 µg/mL. The IC₅₀ value was found to be 31.25 µg/mL.

As lupeol exhibited notable biological activities, antigenotoxic effect of lupeol was assessed using golden Syrian hamsters. The total number of 24 animals was divided into four groups and each group contained six animals. Group 1 animals were served as control. Group 3 animals were pretreated with Lupeol (50 mg/kg b.w. p.o.) for 5 days and were intraperitoneally injected with DMBA (30 mg/kg b.w.), on 5th day after 2hrs of administration of Lupeol. Group 2 animals were given intraperitoneal injection of DMBA (30 mg/kg b.w.) on 5th day. Group 4 animals were pretreated with Lupeol (50 mg/kg b.w. p.o) alone for 5 days and did not receive DMBA. All the animals were sacrificed after 24 hrs of DMBA injection by cervical dislocation for the assessment of micronucleus frequency and DNA damage. Comet assay was performed following the method of Tice et al., 2000, using the fermer bone marrow cells of control and treated animals to assess the antigenotoxic effect of lupeol. Oral pretreatment of Lupeol (50 mg/kg bw) to DMBA treated hamsters significantly suppressed the appearance of tail in the comet and percentage of DNA in tail. The results suggested the potent antigenotoxic effect of lupeol, as evidenced by less damaged DNA, in the bone marrow cells of DMBA treated hamsters (i.e.) The cancerous changes induced by DMBA was suppressed by lupeol.

The phytochemicals present in sesbania grandiflora leaves using the solvents petroleum ether, chloroform, methanol, ethanol and acetone were carried out. The methanol extract possess higher pharmacological potential than other extracts. The phytochemicals present in all extracts are estimated and the chemical components
present in methanol extract are isolated by column chromatography. Among seven compounds isolated, three compounds are having remarkable biological activities. Hence the structure of the biologically active components are evaluated using spectral data. The biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer activities and antigenotoxic effect were studied. The three components out of seven possess remarkable activities. These compounds can be used as therapeutic agents for many diseases. The study proved traditional health claims regarding this plant to be true.