



## Location specific phytochemical and antibacterial analysis of methanolic extracts of *Catunaregam spinosa* (Thunb.) Triveng

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### Abstract

*Catunaregam spinosa* (Thunb.) Triveng, known locally as Gela, belongs to Rubiaceae family. It is used in Ayurveda to treat various ailments. Secondary metabolites from this plant has been extensively characterized and studied. *Catunaregam spinosa* (Thunb.) Triveng plants from two different geographical locations, one from a tribal settlement area in North Goa(rural) and another from SGNP, Mumbai(urban), were sampled to understand the effect of geographical locations on the plant's phytochemical composition. A preliminary phytochemical analysis of total phenolics and flavonoid, along with the antimicrobial activity from leaf and stem samples of both the plants were studied. The leaf sample from Goa showed highest concentration of total phenolics whereas, Mumbai leaf sample showed high flavonoid content and antimicrobial activity with MIC of 18.22mg/ml against *Staphylococcus aureus*. Thus the two plants from different geographical locations showed comparable difference in some of the phyto constituents and also differed in their antimicrobial activity.

**Keywords:** flavonoids, antimicrobial activity, phenolics, gela

### Introduction

Plants are a rich source of medicinally significant compounds. With the rise in antibiotic resistant strains and related diseases, there is a rising demand for herbal medicines as their ingredients are natural and with little or no side effects. The medicinal property of the plant is due to its phytochemical composition. Geographical location plays a very important role in determining the phytochemical composition in a plant, for example: weather conditions (rainy/ windy/drought), soil nutrients, temperature, cultivation practices etc.

Tribes are known to use the plants in their vicinity for various medicinal purposes. The term broadly classified as Ethnobotany, reaches out to conserve the tribe's knowledge of uses of certain plants. Ethnobotanical surveys thus explore plants with medicinal values growing in the forest. Forests experience harsh but natural environmental conditions, but are mostly free from anthropogenic activities. Similar plants also exist in Urban areas, but there is a difference in their ecotype. Moreover, the urban plant may face more challenges than its rural counterpart, with respect to air, water and soil pollution. As a response, they might produce secondary metabolites which might differ both qualitatively and quantitatively from their rural counterpart.

Phytochemical differences in plants which are located in different locations are often observed by researchers. In one study, *Hemidesmus indicus* was sampled from 6 different locations across the Telangana state. Plants from three locations showed maximum number of phytochemicals compared to others [1]. *Moringa oleifera*, collected from four different districts of Tamil Nadu was analysed for its phytochemistry and antioxidant activity. *Moringa oleifera* sampled from Tirunelveli district with loamy red soil, moderate monsoon and less pollution seemed to show highest

anti-oxidant potential and flavonoid content compared to other samples [2]. A study conducted on leaf and tuber of *Tacca leontopetaloides* (L.) Kuntze collected from four geographical locations in Nigeria showed effect of environmental conditions on its phytochemical composition [3]. Three different species of *Pyrola* was studied from different regions in China, showed that a particular *P. calliantha* sample from Left banner of Alxa possessed the strongest antioxidant activity among all. They also suggest that phytochemical methods can be used to identify them from regions of origin [4].

*Catunaregam spinosa* (Thunb.) Triveng, locally known as Gela, was sampled from Satterri, Goa. Various parts of this plant are reported for different ethnobotanical uses, mostly medicinal. It is a large shrub or small tree armed with nearly opposite decussate spines. Spines are 1-3cm long, coming off from above the branchlets. Leaves are 3-6 by 2-3cm, flowers are at the ends of short leaf and Fruit is like a small crab-apple, yellowish, globose, crowned with large calyx limb [5].

*Catunaregam spinosa* (Thunb.) Triveng has been studied extensively for its phytochemical and pharmacological properties. Different extracts of this plant show presence of glycosides, carbohydrates, phytosterols/ triterpenoids, saponins, tannins, phenolics, fixed oils & fats [6]. A new dihydroisocoumarin, a new iridoid 3- deoxyartselaenin and six other compound's structures were elucidated by spectroscopic analysis and NMR data from the bark of this plant [7]. HPTLC analysis of the root bark of *Catunaregam spinosa* (Thunb.) Triveng revealed the presence of oleanolic acid glycoside [8]. Five triterpenoids have been elucidated from *Catunaregam spinosa* (Thunb.) Triveng which has antifeedant activity and toxicity [9]. The plant extract shows antimicrobial activity against pathogens like *Staphylococcus aureus*, *Streptococcus*

*mutans*, *Aspergillus niger* and *Candida albicans* [10]. The fruit and bark DMSO extracts show good antiviral activity against Herpes simplex 1 & 2, vaccinia virus etc. [11]. Previous studies on anti- microbial activity of fruit and leaf of *Catunaregam spinosa* (Thunb.) Triveng have shown inhibition against pathogenic bacteria and fungi.

The objectives of our study was to compare phytochemicals, qualitative and quantitative detection and estimation of phenolics and flavonoids, along with antimicrobial activity of *Catunaregam spinosa* (Thunb.) Triveng sampled from two geographically isolated regions, Satter, Goa and Mumbai.

## Materials and Methods

The ethnobotanical survey was carried out in Satter, Goa where Mr. Ramakant Goankar and his Father Mr. Suryakanth Gaonkar, forest dwellers, were interviewed. The fruits of Gela or *Catunaregam spinosa* (Thunb.) Triveng, are used to treat jaundice by making a concoction of the same. Similarly, it's urban counterpart was collected from Sanjay Gandhi National Park at Mumbai. Both plants were submitted to St. Xavier's Blatter Herberium for authentication.

## Sample Preparation

The leaves and the stem of plants were separated and air dried following which it was oven dried at 55°C till a constant weight was obtained. The samples were ground and stored in dark conditions. The powdered samples were then subjected to maceration with different solvents as per the requirement of the experiment.

## Phytochemical Screening

The leaf and stem samples of both the plants were subjected to qualitative analysis to check for the presence of antimicrobial class of compounds. The tests were as follows: Flavonoid detection by Alkaline reagent test, Phenolic compound detection by lead acetate test, Terpenoids and steroids detection by Salkowski's test, Alkaloids detection by Mayer's test [12] Tannin detection by Ferric chloride test [13] and Glycosides detection by Killer Killiani test [14].

## Quantification Studies

### A. Flavonoid Estimation

To determine the total flavonoid content, Aluminium chloride method of estimation was followed. Different concentrations of Quercetin were used to obtain the standard calibration curve. 1mg/ml of Quercetin was made in Methanol which was further diluted to obtain the following concentrations- 6.25, 12.5, 25, 50, 80 & 100ug/ml. For test, 0.5g of plant dried powder was subjected to cold maceration at 4°C for 16 hours in 70% Ethanol, following which, the supernatant was used for quantification. A volume of 0.5ml of test and control sample was mixed with 1.5ml of Methanol, 0.1ml of 10% Aluminium Chloride, 0.1ml of Potassium acetate and 2.8ml of Distilled water. The blanks for each tube was also prepared, in which 0.1ml 10%aluminium chloride was eliminated and replaced with 2.9ml of Distilled water. The reaction mixture was vortexed and absorbance was measured at 415nm using Double beam UV Visible Spectrophotometer [15, 16, 17].

### B. Total Phenolics estimation

Total phenolics content was determined by Folin Ciocalteu method. 0.5g of the dried powdered sample was macerated with 5ml of Methanol for 2 hours at RT on shaker after which the supernatant was used for estimation. For standard Gallic acid calibration curve, stock of 1mg/ml of Gallic acid was prepared in methanol which was further diluted to obtain different concentrations of gallic acid (50, 80,100,150,200,250,300 & 400 ug/ml). A volume of 0.5ml of test and standard was mixed with 2 ml of 1:10 diluted Folin ciocalteu reagent, followed by 4ml of 7.5% sodium carbonate solution. It was mixed and incubated at RT for 30 minutes, following which it's absorbance was measured at 765nm using Double beam UV Visible spectrophotometer [18, 19].

### C. Determination of anti-microbial activity by ditch plate technique and mic by microdilution method

To determine the anti- microbial effect of the leaf and stem extracts, 1g of dried powder was mixed with 10ml Methanol and macerated for 2 hours at RT on shaker after which the supernatant was collected, evaporated and the extract was dissolved in 1ml of 10% DMSO. For Ditch Plate Technique, 6cm by 1cm ditch was made in the center of sterile MH agar plate into which 1ml of 1g extract in 10% DMSO +2ml of sterile molten MH agar was poured and allowed to solidify. For culture preparation, 18- 24 hour old cultures of *Staphylococcus aureus*, *Escherichia coli*, *Corynebacterium diptherae* and *Pseudomonas aeruginosa* were adjusted to 0.1 OD at 540nm. Each of these were streaked across the ditch on MH agar plate and incubated for 24 hours at 37°C. Following incubation, growth of the organisms was checked for on and around the ditch [20]

For MIC, 1g/ml of extract was prepared the same way in 10% DMSO as for Ditch Plate Technique. The extract was diluted 1:1 in sterile MHB (Muellar Hinton Broth). For Culture preparation, 18-24 hour old culture was adjusted to 0.1OD at 540nm, followed by dilution of this 1:100 in Sterile MHB. On an ELISA Microplate, add 50 microlitre from well 2-8. Add 100 microlitre of the 1:1 MHB diluted extract to Well No.1 in all rows. From this well, pipette out 50 microlitre into the well 2, mix well and transfer 50 microlitre from well 2 to 3 and so on till Well No.7. Discard 50 microlitre from well 7. Now add 50 microlitre of 1:100 diluted culture to all the wells. Well no 8 acts as positive control. 1mg/ml Ampicillin was used for antibiotic control, 10% DMSO was used as inhibitory control and the extract itself was used as negative control (no culture). The experiment was performed in triplicates. After addition of culture, the plate was incubated at 37°C for 24 hours. After incubation, 30 micro litre of 0.015% Resazurin indicator dye was added to each well. Wells remaining blue was assessed as 'No growth' and wells showing Pink colouration was assessed as 'Growth'. The dilution at which the well-turned pink was determined as it's MIC [21]

## Results

### A. Phytochemical screening

The results of the phytochemical tests to detect the presence of different class of compounds are as follows (Table 1):

Table 1

Class of Compounds	Leaf Sample		Stem Sample	
	Goa	Mumbai	Goa	Mumbai
Flavonoids	+	+	+	+
Phenolics	+	+	+	+
Tannins	+	+	+	+
Steroids	+	-	+	+
Terpenoids	+	+	+	+
Alkaloids	+	+	+	+

Key: + -- absence

**B. Quantification Studies**

The concentration of phenolics from leaf and stem of *Catunaregam spinosa* (Thunb.) Triveng from Goa and Mumbai was measured using a standard calibration graph of Gallic acid (Figure 1). The results of the experiment performed in triplicates are reported in mg GAE/ gram of plant extract.

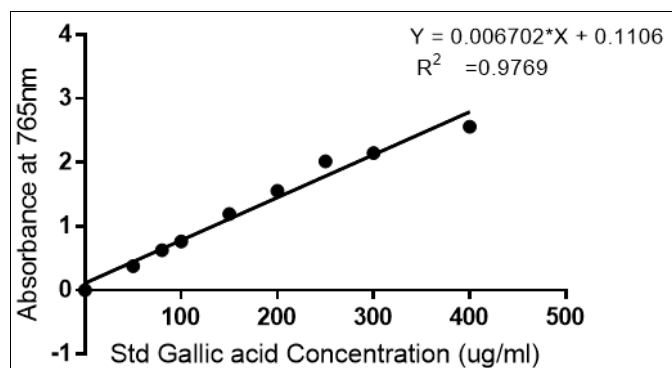


Fig 1: Standard Gallic acid Vs Absorbance Graph

Similarly, the concentration of flavonoids from leaf and stem of *Catunaregam spinosa* (Thunb.) Triveng from Goa and Mumbai was measured using a standard calibration graph of Quercetin (Figure 2). The experiments were performed in triplicates and the results are reported in mg/gram of plant extract.

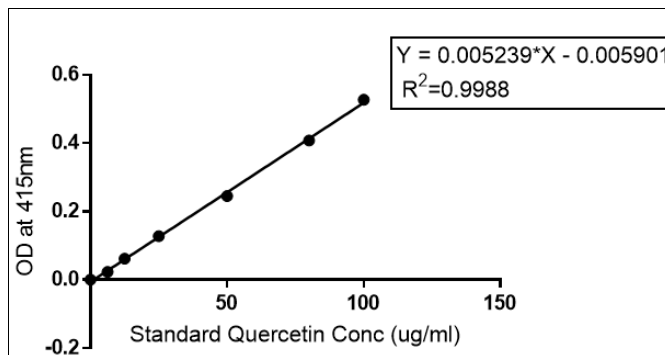


Fig 2: Quercetin Standard Curve Concentration Vs Absorbance Graph

The comparative flavonoid (Figure 3) and phenolics (Figure 4) content in leaf and stem of *Catunaregam spinosa* (Thunb.) Triveng from two distinct geographical locations are represented in the graph below.

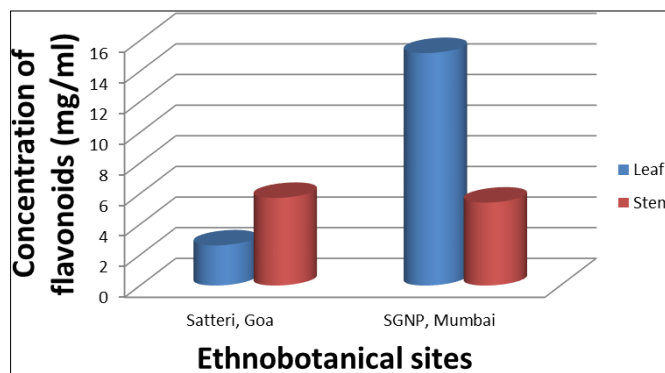


Fig 3: Comparison of flavonoid content in *Catunaregam spinosa* (Thunb.) Triveng from Goa and Mumbai

The flavonoid content in the *Catunaregam spinosa* (Thunb.) Triveng leaf and stem from Goa were found to be  $2.6225 \pm 0.991$  and  $5.7219 \pm 0.232$  mg/gram of extract (in QE) respectively. Whereas, flavonoid content in leaf and stem of the same plant from Mumbai was found to be  $15.1810 \pm 0.932$  and  $5.4166 \pm 1.705$  mg/gram of extract (in QE) respectively.

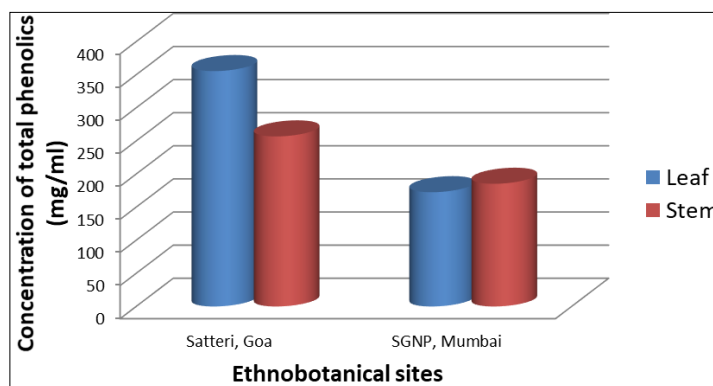


Fig 4: Comparison of total phenolics content in *Catunaregam spinosa* (Thunb.) Triveng from Goa and Mumbai

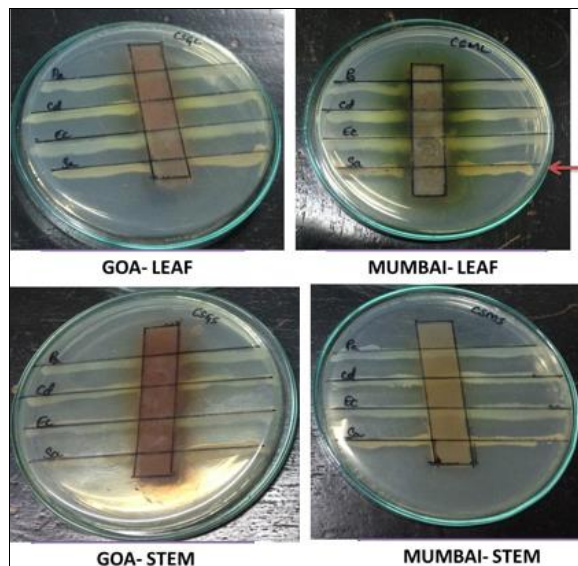
The phenolics content in the *Catunaregam spinosa* (Thunb.) Triveng leaf and stem from Goa were found to be  $355.569 \pm 25.21$  and  $256.902 \pm 54.357$  mg/gram of extract (in

GAE) respectively. Whereas, phenolics content in leaf and stem of the same plant from Mumbai was found to be  $172.577 \pm 7.712$  and  $185.335 \pm 3.793$  mg/gram of extract (in

GAE) respectively.

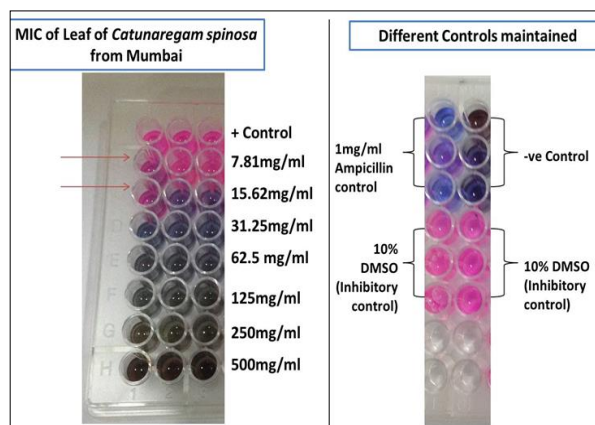
### C. Antimicrobial activity and MIC

Ditch plate technique was performed with leaf and stem samples of *Catunaregam spinosa* (Thunb.) Triveng from Goa and Mumbai. Amongst the four samples, only the Mumbai Leaf sample inhibited the growth of *Staphylococcus aureus*. All other samples seemed to show no anti- microbial activity. It not only inhibited the growth on the ditch but also around it as seen in Figure 5.



**Fig 5:** Comparison of anti- microbial activity of Leaf and Stem extracts of *Catunaregam spinosa* (Thunb.) Triveng from Goa and Mumbai.

MIC of *Catunaregam spinosa* (Thunb.) Triveng Leaf from Mumbai was performed by Micro dilution technique, where the effect different concentrations of the extract (500mg/ml to 7.81mg/ml) was tested against *Staphylococcus aureus*. It was found that 18.22mg/ml of extract was required for complete inhibition of growth *Staphylococcus aureus*. Positive control, Inhibitory control (DMSO) showed growth whereas, Negative control and Antibiotic control (1mg/ml) Ampicillin did not show any growth of the organism.



**Fig 6:** MIC by microdilution technique on ELISA microplates using resazurin indicator

### Discussion

The ethnobotanical survey at Satterri, Goa documented the medicinal use of 43 plants. Although many plants were sampled, only 4 could be authenticated. Goa region is rich in biodiversity and ethno botanical surveys have been conducted, which are mostly restricted to South Goa. Sattari, belongs to North Goa, where many medicinal plants have also been documented previously. According to a study conducted in 2014, 130 medicinal plants were documented, which the “Vaid” or traditional healer had described [22]. Sattari also harbours many “Devrai”s or Sacred Grove, which grows medicinally important plants, under protective environment that is restricted for common people. Therefore, there is a great scope for conducting many more ethno botanical surveys in such areas. The medicinal property of the fruit of *Catunaregam spinosa* (Thunb.) Triveng, for treatment of Jaundice is being reported for the first time. Every part of *Catunaregam spinosa* (Thunb.) Triveng has been reported to have a medicinal property, which includes fruit, leaf, bark, root and stem.

The aim of the study was to analyse differences in phytochemical compositions of the same plant, sampled from different geographical locations. Sattari, is a well conserved region of North Goa, that is rich in flora and fauna and also protected from anthropogenic activities. Mumbai on the other hand is a metropolitan urban city, with its environmental challenges and pressures. Different environmental factors affect the constituents and it’s quantity in the plants. While studying the Total phenol content, phytochemical diversity and in vitro antioxidant activity of *Aloe vera* from different states of India, it was found that different climatic conditions have different effects of this plant’s constituents [23]. Variation in phytochemical composition of *Pterocarpus osum* was observed which was sampled from different geographical location having different climatic conditions [24]. The results of phytochemical screening revealed no significant differences in the secondary metabolite constituents. But differences in the quantities of Total phenolics and Total flavonoid content were observed between the plants from Sattari as compared to Mumbai. The total phenolics in Leaf and Stem extracts of *Catunaregam spinosa* (Thunb.) Triveng from Sattari was higher than that of Mumbai; however, the total flavonoid content in contrary was highest in the leaf extract of *Catunaregam spinosa* (Thunb.) Triveng from Mumbai. Phenolic compounds in plants are those secondary metabolite group which consist of a wide variety of molecules with heterogenous structure, having an aromatic ring bearing one or more hydroxyl substituents. Flavanoids occupies a major class under phenolics. The reason for *Catunaregam spinosa* (Thunb.) Triveng from Goa to have high phenolic content but relatively low flavonoid content might be due to presence of other major phenolics like Tannins, Lignins, Quinones etc., The differences in the quantity suggests the effect of environmental factors like climate, soil nutrients, temperature on these constituents.

*Catunaregam spinosa* (Thunb.) Triveng leaf from Mumbai showed significant anti- microbial activity against *Staphylococcus aureus*, with a low MIC. There can be a correlation between high flavonoid content and anti- microbial

activity in this plant. Many flavonoids have been reported to have anti-microbial activity, for example, robinetin, myricetin, apigenin, rutin etc<sup>[25]</sup>. Also, fruits of this plant from different parts of India has previously been reported to have anti-microbial activity against Gram positive organisms, Gram negative organisms and Fungi<sup>[10, 26, 27]</sup>. One study conducted with leaf hexane extract of *Catunaregam spinosa* (Thunb.) Triveng, showed MIC as low as 0.16mg/ml against *Staphylococcus aureus*, indicating that the anti-microbial compound maybe non-polar in nature<sup>[28]</sup>. Even the full plant methanolic extract showed anti-microbial activity against certain Gram positive and Gram negative organisms<sup>[29]</sup>.

This study served the basis for comparison of phytochemical analysis of *Catunaregam spinosa* (Thunb.) Triveng, sampled from Sattari with that sampled from Mumbai. There is a significant difference in phenolics and flavonoids content and they also differ in their antimicrobial activity. The difference can be attributed to the environmental factors due to different geographical locations. Thus this study would help a researcher to predict location specific phytochemical composition that would help in plant selection. In this case, the *Catunaregam spinosa* (Thunb.) Triveng leaves from Mumbai location, show high flavonoid content and a high antimicrobial activity and *Catunaregam spinosa* (Thunb.) Triveng whereas leaves from Satterri, Goa, have higher phenolic content.

An extended comparative study can be conducted by determining different chemical functional groups of these two plant extract by HPTLC, GC-MS and NMR. Bioactive component can be determined by anti-microbial analysis, cyto-toxicity testing etc., which is indicative of the use of plant parts in bio-formulations.

## References

1. Suman Kumar R, Ramchandra Reddy P, Gangadhar Rao S, Nethaji K. A comparative study of phytochemical screening in plant *Hemidesmus indicus* (L.) R.Br. collected from different geographical regions of Telangana state, Asian Science. 2016; 11(1):47-51.
2. Inbathamizh L, Dr. Padmini E. Effect of geographical properties on the phytochemical composition and anti-oxidant potential of *Moringa oleifera* flowers, BioMedRx. 2013; 1(3):239-247.
3. Borokini TI, Ayodele AE. Phytochemical screening of *Tacca Leontopetaloides* (L.) Kuntze collected from four geographical locations in Nigeria, International Journal of Modern Botany. 2012; 2(4):97-102.
4. Dongmei Wang, Fengyuan He, Zhenjiang Lv, Dengwu Li. Phytochemical composition, anti-oxidant activity and HPLC fingerprinting profiles of three *Pyrola* species from different regions, PLOS one. 2014; 9(5).
5. Almeida M. Flora of Maharashtra (Rubiaceae to Ehretiaceae). Mumbai: St. Xavier's college. 2001, 301-464.
6. Surabhi Shrivastava Leelavathi S. Preliminary phytochemical evaluation of leaf extracts of *Catunaregam spinosa* (Thunb.) Triveng Thunb, International journal of pharmaceutical sciences review and research. 2010; 3(2):114-118.
7. Guangchun Gao Shuhua Qi, Si Zhang Hao Yin, Zhihui Xiao Minyi Li, Qingxin Li. Minor compounds from the stem bark of Chinese mangrove associate *Catunaregam spinosa* (Thunb.) Triveng, Pharmazie. 2008; 63:542-544.
8. Varadharajan Madhavan, Sunkam Yoganarasimhan, Magadi Gurudeva, Rajamanickam Deveswaran, Deepti Reddy. Vedavathi Bankapura, Pharmacognostical studies on the root bark and stem bark of *Catunaregam spinosa* (Thunb.) Triveng (Thunb.) Tiruv. (Madanaphala)- an Ayurvedic drug, Spatula DD. 2014; 4(2):89-99.
9. Kedi Yang, Yuejuan Li, Li GE, Zuzeng Qin, Isolation of triterpenoids from *Catunaregam spinosa* (Thunb.) Triveng, Advanced Materials Research. 2011, 236-238, 1731- 1737.
10. Tiloo SK, Bodhankar MM, Waikar SB and Pande VB. Antimicrobial activity of formulation containing bioactive extract of endocarp of *Randia spinosa*, International journal of advances in pharmacy, biology and chemistry. 2012; 1(2):172-175.
11. Parmar KA, Prajapati SN, Jani AP. Phytochemical screening of *Randia dumetorum* plant extracts for the antioxidant activity by DPPH method and their antibacterial and antiviral activities, International journal of chemical sciences. 2010; 8(2):1101-1109.
12. Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG. Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Olea dioica* Roxb. infected with the rust fungus *Zaghouania oleae* (E. J. Butler) Cummins and non-infected plants, Journal of Pharmacognosy and Phytochemistry. 2014; 3(4):69-72.
13. Philip Pizzolato, Lillie RD. Mayer tannic acid- ferric chloride stain for mucins, The journal of Histomchemistry and cytochemistry. 1973; 21(1):56-64.
14. Dr. Rimjhim Sheel, Kumari Nisha and Prof. Jainendra Kumar, Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*, IOSR Journal of Applied Chemistry. 2014; 7(1):10-13.
15. Akbay P, Basaran AA, Undeger U, Basaran N. In vitro immunomodulatory activity of flavonoid glycosides from *Urtica dioica*. Phytoter. Res. 2003; 17:34-37.
16. Kaufman PB, Leland Cseke J, Ara Kirakosyan, Sara L. Warber, James A. Duke and Harry L. Briemann, Natural products from plants. New York CRC press, 1999.
17. Bhaigyabati Th P, Grihanjali Devi GC. Bag Total Flavonoid Content and Antioxidant Activity of Aqueous Rhizome Extract of Three *Hedychium* Species of Manipur Valley. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014; 5(5):970.
18. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin- Ciocalteu reagent. Nat Protoc. 2007; 2(4):875-877.
19. Fatma Alhakmani, Sokindra Kumar, Shah Alam Khan. Estimation of total phenolic content, in-vitro antioxidant and antiinflammatory activity of flowers of *Moringa oleifera*. Asian Pacific Journal of Tropical Biomedicine, 2013; 3(8):623-627.
20. Rice WG, Lonergan AM. Ditch- plate method for testing bacterial resistance to antibiotics, American Journal of Clinical Pathology. 1950; 20(1):68-70.

21. Mounyr Balouiri, Moulay Sadiki, Saad Koraichi Ibsouda. Methods for in vitro evaluating antimicrobial activity: A review, *Journal of Pharmaceutical Analysis*. 2016; 6(2):71-79.
22. Lata Satyawan Naik Puttaiah ET, Ananth Nag B. Ethnobotanical studies of some plants included in folk medicines of Goa, *International Journal of Basic and Applied Sciences*. 2014; 3(1):6-13.
23. Sandeep Kumar, Amita Yadav, Manila Yadav, Jaya Prakash Yadav. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.) Burm. f., *BMC Research Notes*, 2017; 10:60.
24. Orishadipe Abayomi, Fatokun Olakunle, Okolo Simon, Akanji Fausat and Thomas Sunday, Comparative composition of the leaf extracts of *Pterocarpus osun* from different geographical regions of Nigeria, *American Journal of Ethnomedicine*. 2015; 2(5):297-302.
25. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids, *International Journal of Antimicrobial Agents*. 2006; 27(2):181.
26. Anand SP, Deborah S, Velmurugan G. Antimicrobial activity, nutritional profile and phytochemical screening of wild edible fruit of *Catunaregam spinosa* (Thunb.) Triveng (Thunb.) Triveng, *The Pharma Innovation Journal*. 2017; 6(10):106-109.
27. Movalia Dharmishtha, Gajera Falguni, Antibacterial activity of methanolic fruit extract of *Randia dumetorum* Lamk, *International Journal of Pharma Tech Research*. 2009; 1(3):679-681.
28. Yik Sin Chan, Bee Ling Chuah, Wei Quan Chan, Ri Jin Cheng, Yan Hang Oon, Kong Soo Khoo, Nam Weng Sit, Evaluation of Medicinal Plants, *Catunaregam spinosa* (Thunb.) Triveng, *Houttuynia cordata*, and *Rhapis excelsa* from Malaysia for Antibacterial, Antifungal and Antiviral Properties, *World Academy of Science, Engineering and Technology*. 2014; 8(11).
29. Nishanta Rajakaruna, Cory S, Harris GHN. Towers, Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka, *Pharmaceutical Biology*. 2002; 40(3):235-244.