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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *Gymnema sylvestre*: A MEDICINAL PLANT

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ABSTRACT

The present study was carried out to evaluate the phytochemical and antimicrobial activity of *Gymnema sylvestre* against ten microbial strains causing oral infections. The qualitative phytochemical analyses were carried out following the Indian pharmacopoeia and the methods. The MIC values of the plant extracts were determined against the selected test organisms using the methods as described by National Committee for Chemical Laboratory Standard and the in vitro antimicrobial activity was determined by using the agar disc diffusion method. The phytochemical analysis carried out revealed the presence of alkaloids, phenolic compounds, flavonoids, glycosides, tannins and tri terpenoids in this medicinal plant. The antimicrobial activity of five different extracts of medicinal plants were evaluated using well diffusion method against *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Salmonella typhi*, *Chromobacterium violaceum*, *Burkolderia mallei* and *Candida albicans* respectively. The chloroform extracts of this plant shown best antimicrobial activity against selected microbes. The results provide justification for the use of the medicinal plants to treat various oral infections.

KEYWORDS: *Gymnema sylvestre*, Diabetic, Chloroform Extract, Bioactive Compounds, Antimicrobial Activity

Medicinal plants have played a critical role in the health and well-being of humans both prophylactically and therapeutically for controlling diseases (Upadhyay *et al*. 2014). *Gymnema sylvestre* is important medicinal plant and it is commonly called as "Gudmar" (gud-jaggery, mar-kills). The plant is slow growing large woody climber. The genus *Gymnema* belongs to the family Asclepiadaceae (Pushpa and Mahesh, 2017). *Gymnema sylvestre* has been under the use to treat diabetes over several years. Leaves are used for the preparation of decoction and used for the treatment of diabetics. This plant was used as remedy for rheumatism, cough, ulcer, jaundice, dyspepsia, constipation, eye irritation and snakebite. It was also used as an anodyne, digestive and liver tonics, diuretic, stomachic, laxative, appetite suppressant, stimulant, anti-helminthic, cardiotonic, expectorant, antipyretic and uterine tonic (Mahajan *et al*. 2011). Gymnemic acid present in the leaves is believed to reduce blood glucose level. Leaves and roots are also used to treat headache, polyuria, leprosy, wounds and prurutis. The raw drug is mainly collected from the wild. It is reported to cure cough, dyspnoea, ulcers, *pitta, kapha* and pain in the eyes. The fresh leaves when chewed have the remarkable property of paralysing the sense of taste for sweet and bitter substance for some time (Warrier *et al*., 1995). The drug is described as a destroyer of *madhumeha* (glycosuria) and other urinary disorders. Root has long been reputed as a remedy for snakebite.

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MATERIALS AND METHODS

Plant Collection

Plant *Gymnema sylvestre* was collected from different localities in and around Madurai and maintained in the herbal garden of Saraswathi Narayanan College (Autonomous), Madurai, Tamil Nadu, India. The plant was identified and authenticated by referring the standard taxonomic characteristic features (keys) according to the Flora of Madras Presidency (Gamble, 1935) and the Flora of Tamil Nadu Carnatic (Mathew, 1991). The voucher specimens of the plants and photographs were kept in the Department of Botany, Saraswathi Narayanan College (Autonomous), Madurai, Tamil Nadu, India for future reference.

Preparation of Extracts

The entire plant of *Ceropegia juncea* was used for preparation of extracts. The plant material was collected, cut into small pieces, washed in water and dried at 40 ºC. The dried plant pieces were then grind in mechanical grinder. The powder was sieved using a mesh sieve and stored in air tight bottles. 50 g of the plant was taken in a Soxhlet's apparatus. The following series of solvents were used for the extraction: petroleum ether, chloroform, ethyl acetate and methanol. All solvents (250 ml) used were of analytical grade (AR). The extraction carried was of hot

type and was for about 48 hours in each solvent. Before the successive solvent extraction, each time the powdered material was air dried below 50 ºC. To prepare aqueous extract fresh plant material (50 g) was harvested and used. The plant material was surface disinfected with 0.1 % (w/v) $HgCl_2$ solution for 5 min. It was washed thrice with sterilized distilled water for 5 min. each time. The plant material was ground with mortar and pestle in 100 ml sterile distilled water. The homogenized tissue was centrifuged at 3500 rpm for 20 min. The supernatant was taken as aqueous extract. All the extracts were concentrated by distillation of the solvent and evaporating to dryness using a flash evaporator. After complete solvent evaporation, each of the solvent extract was weighed and preserved at 5 ºC in air tight bottles until further use.

Growth and Maintenance of Test Microorganisms

The Gram positive stains of *Staphylococcus aureus* and *Streptococcus faecalis* and Gram negative strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Salmonella typhi*, *Chromobacterium violaceus* and *Burkolderia mallei* were maintained at 37^{O}C in Nutrient Broth and the fungus *Candida albicans* was maintained in Potato dextrose agar medium until the preparation of inoculum.

Inoculum Preparation

All the test bacterial species were maintained in nutrient agar media. 36 hours old bacterial cultures were inoculated into nutrient broth and incubated at 35 ± 2^{o}C on a rotary shaker (Remi, India) at 100 rpm. After 36 h incubation, the bacterial suspension was centrifuged at 10,000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1 x 10^8 cfu / ml using UV-visible spectrophotometer (Hitachi U-2000, Japan) by reading the OD of the solution to 0.45 (A_{610} nm) (Basri and Fan, 2005). Fungal colonies were harvested from 9-10 day old cultures, which were maintained on potato dextrose agar (PDA) medium. The spores were suspended in sterile distilled water and the spore suspensions were adjusted to 1 x 10^6 spores/ ml by counting with a haemocytometer (Dayang *et al*., 2005).

Phytochemical Screening

The qualitative phytochemical analyses were carried out following the Indian pharmacopoeia and the methods described by Harborne (1973). The five different solvent extracts obtained by successive solvent extraction were tested separately for the presence of various phytoconstituents namely alkaloids, amino acids, carbohydrates, fats and fixed oils, flavanoids, glycosides, saponins, gums, lignins, proteins, steroids, triterpenoids, tannins and phenolic compounds.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of the plant extracts were determined against the selected test organisms using the methods as described by National Committee for Chemical Laboratory Standard (1993). The extracts were added aseptically to sterile Mueller–Hinton (MH) broth and potato dextrose agar (PDA) medium separately in appropriate volumes to produce the concentration range of 20-100 mg/ml. The resulting MH and PDA media were immediately poured into Petri plates after vortexing. The plates were inoculated with 100 µl of bacteria (1 x 10^8 cfu/ ml) and fungus (1x10^6 spores /ml)[9]. Amikacin (20-30 µg) was used as a reference antibiotic drug and used as positive control. The paper discs loaded with 50 µl of DMSO and placed in MH and PDA were used as negative control. The inoculated plates were incubated at 37 ^{o}C for 24 h and 37 ^{o}C for 48 h for bacteria and the fungus respectively. At the end of the incubation period, the plates were evaluated for the presence or absence of growth of the test bacteria and fungus. The MIC values were determined as the least concentration of the extracts where absence of growth was recorded. For each extract, five replicates were maintained and each test was repeated at least thrice.

Antimicrobial Activity

In vitro antimicrobial activity was determined by using the agar disc diffusion method (Andrews**,** 2001). Whatman filter paper (No.1) discs of 6 mm diameter were impregnated with 50 µl of the solution of various extracts (at 60 mg /ml) prepared using DMSO. The discs were evaporated at 37^{o}C for 24 hours. The standard antibiotics amikacin discs were prepared as described above using the appropriate concentration (30µg/ml) of the drug. An even spread of microorganisms was prepared by transferring 100 µl of microbial suspension to Mueller-Hinton agar plates for bacteria (1 x 10^8 cfu / ml) and potato dextrose agar plates for the fungus (1 x 10^6 spores/ ml) using sterile cotton buds. The extract discs were then positioned on the inoculated agar surface. Standard 6 mm discs containing amikacin (30µg/ml) were used as positive controls. Negative controls were made using paper discs loaded with 50 µl of DMSO. The plates were then incubated at 37^{o}C

for 24 h for the bacteria. On the other hand, *Candida albicans* was incubated at 37°C for 48 h. The screening for antimicrobial activity was done by measuring the diameter of a clear inhibition zone around the disc. The mean diameter of inhibition zone was measured to the nearest millimeter (mm) based on three readings of the diameter zones of each target microorganism using a vernier caliper. For each extract, five replicates were maintained and each test was repeated at least thrice. Then the results were expressed as mean ± S.E.

RESULTS

Phytochemical Screening

The leaves of *Gymnema sylvestre* subjected to solvent extraction exhibited high extractive value of 3.324% in petroleum ether followed by methanol (3.048%), chloroform (2.136%) and ethyl acetate (1.648%). There was only less than 1% of extractive value (0.74%) while using water. Among various solvent used, positive response for alkaloids, triterpenoids, phenolic compounds, tannins, proteins and aminoacids, steroids and sterols, fixed oil and fat and glycosides were observed in chloroform extract. Presence of alkaloids, carbohydrate, saponin, flavanoids and glycosides were detected in methanolic extract. Similarly, alkaloid, carbohydrate, saponin and phenolic compounds were confirmed in aqueous extract. The leaf extract of ethyl acetate exhibited the presence of alkaloids, phenolic compounds, tannins, flavanoids and glycosides. Moreover, gums and mucilage were absent in all the solvent extracts. The petroleum ether extract had only tri terpenoids and steroids only. In general, alkaloids were found to be present in all the extracts except petroleum ether. The chloroform extract had majority of phytochemical constituents followed by methanolic extract. The phytocompounds alkaloids, flavanoids and glycosides were at moderate level in the leaf extracts prepared with ethyl acetate and methanol (Table 1).

Table 1: Phytochemical screening of crude leaf extracts of *Gymnema sylvestre*

	Phytochemical constituents										
Solvents used	**Alkaloids**	**Carbo Hydrates**	**Saponins**	**Tri terpenoids**	**Phenolic compounds & Tannins**	**Proteins & Amino acids**	**Steroids & Sterols**	**Fixed oil & Fat**	**Flavone & Flavanoids**	**Glycosides**	**Gum & Mucilage**
Petroleum ether	-	-	-	+	-	-	+	+	-	-	-
Chloroform	+	-	-	+	+	+	+	+	-	+	-
Ethyl acetate	++	-	-	-	+	-	-	-	++	++	-
Methanol	++	+	+	-	-	-	-	-	++	++	-
Water	+	+	+	-	+	-	-	-	-	-	-

+++ = maximum; ++ = moderate; + = minimum; - = absent.

Antimicrobial Activity

MIC values of leaf extract of this plant against tested bacteria and a fungus was ranging from 20-80 mg/ml. Petroleum ether extract exhibited less MIC value (20 mg/ml) against *Streptococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniaee* and *Chromobacterium violaceum* in all other organisms, it was effective at MIC between 60-80 mg/ml. The high MIC (80 mg/ml) was noticed for *Proteus vulgaris* and *Chromobacterium violaceum* (60 mg/ml). The minimum inhibitory concentration assay made against selected organisms also confirmed that the MIC values for the solvent extracts of leaf of test plant was higher than standard antibiotics (amikacin at 20-30 µg/ml and ketokonazole at 50 µg/ml). (Table 2).

Table 2: Minimum inhibitory concentration of crude leaf extracts of *Gymnema sylvestre*

MIC (mg/ml)							
Test microorganisms	Gram +/- Bacteria & Fungi	Solvent Extracts			Positive control		
		Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water	Amikacin (µg/ml)
Staphylococcus aureus	+	60.00	40.00	20.00	20.00	80.00	30
Streptococcus faecalis	+	20.00	20.00	20.00	60.00	80.00	30
Escherichia coli	-	20.00	40.00	20.00	20.00	80.00	30
Pseudomonas aeruginosa	-	60.00	20.00	20.00	20.00	80.00	30
Klebsiella pneumonia	-	20.00	20.00	60.00	60.00	80.00	25
Proteus vulgaris	-	80.00	80.00	60.00	60.00	80.00	30
Solmonella typhi	-	60.00	40.00	20.00	20.00	80.00	20
Chromobacterium violaceum	-	20.00	60.00	40.00	40.00	20.00	30
Burkolderia mallei	-	80.00	40.00	60.00	60.00	-	25
Candida albicans	Fungus	80.00	20.00	40.00	20.00	80.00	(Ketokonazole 50 µg/ml)

Values are the average of at least three determinations

- = No inhibition

Table 3: Antimicrobial activity of crude leaf extracts of *Gymnema sylvestre*

Zone of inhibition (mm)							
Test microorganisms	Gram +/- Bacteria & Fungi	Solvent Extracts			Positive control		
		Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water	Amikacin (30 µg/ml) / Ketakonazole (50 µg/ml)
Staphylococcus aureus	+	05.47±0.10a	18.75±0.10a	10.05±0.10 b	12.98±0.08b	07.92±0.10a	17.03±0.10b
Streptococcus faecalis	+	10.10±0.09c	20.05±0.10c	14.02±0.08 a	09.27±0.10a b	08.62±0.10a b	19.02±0.08c
Escherichia coli	-	07.95±0.10a b	17.04±0.08b	09.95±0.10 a	10.05±0.10b	05.12±0.10a	18.90±0.0c
Pseudomonas aeruginosa	-	06.02±0.10a b	20.04±0.09c	08.05±0.10 a	10.05±0.10b	09.51±0.10a	17.05±0.10b
Klebsiella pneumonia	-	10.02±0.10c	20.05±0.10c	05.92±0.10 a	08.98±0.10	04.76±0.10a	17.95±0.10b
Proteus vulgaris	-	05.87±0.10a	05.92±0.10a	07.26±0.10 a	07.86±0.10	06.62±0.10a b	16.95±0.09a
Solmonella typhi	-	03.69±0.10a	17.05±0.10b	10.05±0.10 b	12.05±0.10c	08.31±0.10b	17.95±0.10b
Chromobacterium violaceum	-	13.05±0.10e	26.05±0.10d	17.05±0.10 c	16.03±0.09d	10.04±0.10c	19.05±0.10c
Burkolderia mallei	-	07.12±0.10a	18.05±0.10b c	08.01±0.10 b	09.13±0.10b	00.00±0.00a	20.06±0.10d
Candida albicans	Fungus	06.92±0.10a b	20.05±0.10c	17.05±0.10 c	10.05±0.10b c	06.47±0.10a b	16.94±0.10a

Values represent mean ±SE. Mean followed by the same letter within columns are not significantly different (P=0.05) using Duncan's multiple range test.

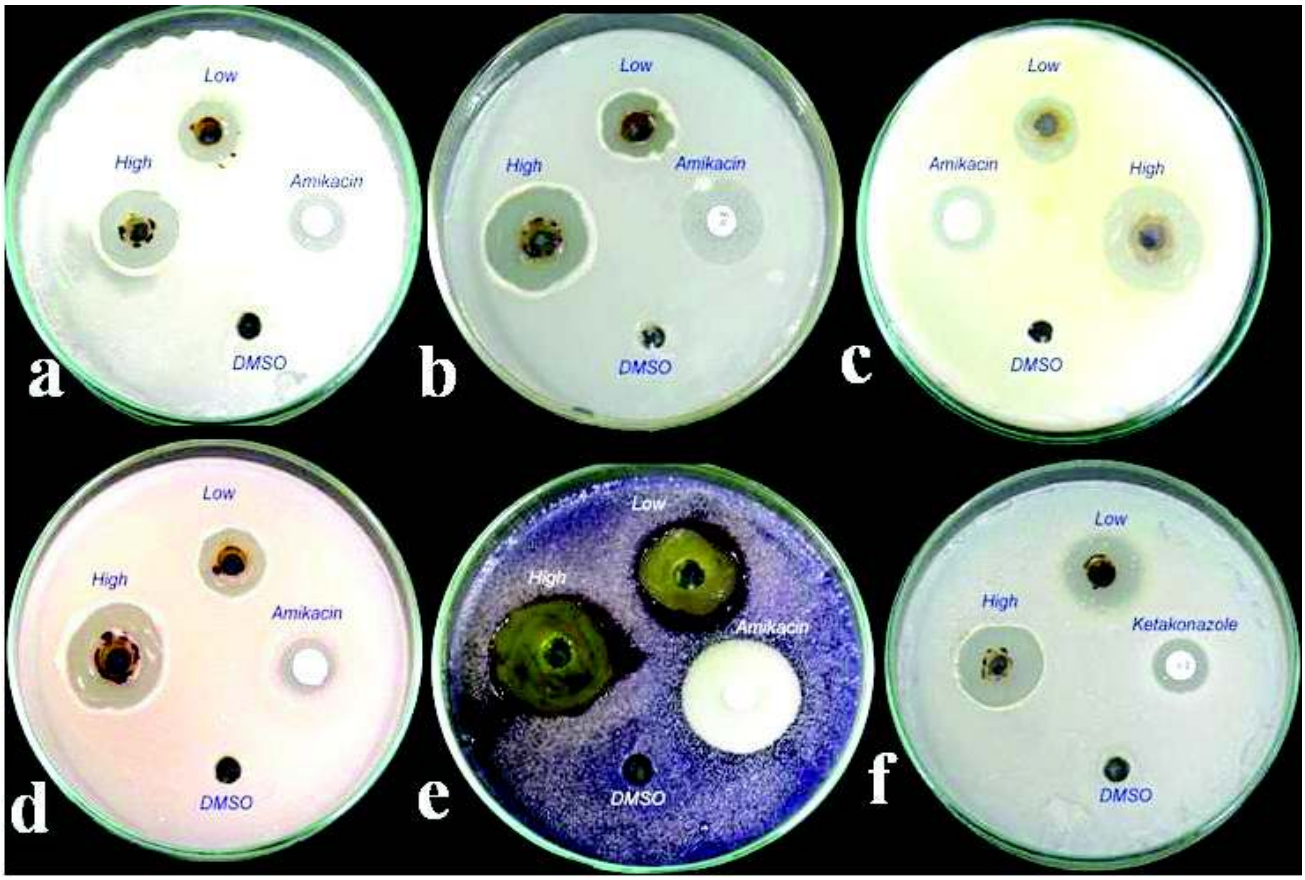

Figure 1: Antimicrobial activity of crude leaf extracts of *Gymnema sylvestre* [a, b, c, d, e & f – zone of inhibition of chloroform extract against *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Chromobacterium violaceum* and *Candida albicans*]

Among different solvent extracts, chloroform extract showed significant antimicrobial activity against all tested microorganisms except *Proteus vulgaris*. Further, *Chromobacterium violaceum* (26.05) and *Candida albicans* (20.05 mm) were found to be more susceptible to chloroform extract of *Gymnema sylvestre* than all other solvent extracts. The ethyl acetate extract exhibited inhibition zone of 5.92 mm to 17.05 mm against all microbes tested. It showed significant inhibitory activity only against *Candida albicans* as compared to control. Methanolic extract of *Gymnema sylvestre* had inhibition zone against *Chromobacterium violaceum* (16.03 mm), *Staphylococcus aureus* (12.98 mm), *Escherichia coli* (10.05 mm), *Pseudomonas aeruginosa* (10.05 mm) and *Candida albicans* (10.05 mm). However, the zone of inhibition against all the tested microorganisms was not greater than positive control. It was recorded that the extracts prepared using petroleum ether and aqueous extract had poor inhibition zone for both Gram positive and Gram negative bacteria and a fungus. Comparatively, the chloroform extract of *Gymnema sylvestre* had effective antimicrobial property irrespective of microorganisms used in this study. The Gram positive bacteria *Staphylococcus aureus* and *Streptococcus faecalis* and Gram negative bacteria viz., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Chromobacterium violaceum* were significantly inhibited by chloroform extract. Similarly, the fungus *Candida albicans* was also inhibited greater than positive control (Table 3 & Figure 1).

DISCUSSION

The solvents used for preparation of crude extracts of *Gymnema sylvestre* exhibited maximum extractive value in petroleum ether**.** In earlier studies, the high extractive value of methanol for woody stem extract of *Wrightia tinctoria* and ethanol for whole plant extract of *Cardiospermum halicacabum* was reported. The antibacterial efficiency of methanoic and aqueous extracts of *Gymnema sylvestre*, *Terminalia arjuna* and *Psidium guajava* was also reported against those test Gram negative

bacteria (Okwu and Okwu, 2004; Sripathi and Uma Sankari, 2010; Murugan, M and Mohan, 2012)**.** The earlier attempts on qualitative analyses recorded the presence of alkaloid, tannin, steroids and flavanoids in chloroform extract of leaf of *Gymnema sylvestre* (Gajendran *et al*., 2012). The study emphasizes that the solvent methanol might be employed for isolation of phytochemicals from plants. Moreover, among the plants selected, leaf of *Gymnema sylvestre* found to have potential bioactive compounds. It has been reported that the members of family Asclepiadaceae is well known to Indian System of Medicine since ancient time as they contain several phytochemicals likes alkaloids, sterols, tannins, terpenoids and flavanoids (Nikajoo, 2009; Arumugasamy *et al*., 2013).

The methanolic leaf extract of *Gymnema sylvestre* (David and Sudarsanam, 2013). The MIC value was between 20 to 80 mg/ml for bacteria and fungi while using methanolic extracts of *Chelidonium majum* (Ciric *et al*., 2008) and it was ranging from 50 to 80 mg/ml for different methanolic extracts of stem, leaf and root of *Vitellaria paradoxa* (Ndukwe *et al*., 2007). Kishor Naidu *et al*. (2013) reported low MIC values from 15.6 mg/ml to 62.5 mg/ml of methanolic extract of *Gymnema sylvestre* for selected Gram positive and negative bacteria. The methanolic and chloroform extracts of *Gymnema sylvestre* also noticed a high degree of antimicrobial activity against *Escherichia coli* and *Candida albicans* (David and Sundarsanam, 2013), *Escherichia coli* and *Proteus* sp. (Sripathi and Uma sankari, 2010), *Escherichia coli, Serratia marcessens*, *Staphylococcus aureus* and *Candida albicans* (Wani *et al*., 2012) and several Gram negative bacteria and *Staphylococcus aureus* (Kishor Naidu *et al*., 2013). On the other hand, petroleum ether and aqueous extracts of leaf and stem of *Gymnema sylvestre* found effective against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* (Murugan and Mohan, 2012).

CONCLUSION

The leaf extract of different solvents of Gymnema sylvestre shown the potential bioactive compounds like alkaloids, carbohydrates, saponinsm triterpenoids, phenolic compounds, tannins, proteins, steroids, fixed oil, flavanoids and glycosides. The MIC values 20-80 observed from different solvent extracts of *G. sylvestre*. Among different solvent extracts, chloroform extract showed significant antimicrobial activity against all tested microorganisms.

REFERENCES

Andrews J.M, 2001. The development of the BSAC standardized method of disc diffusion testing. J. Antimicrob. Chemother, **48** (Suppl. 1):29-42.

Arumugasamy Karupannan, Madathupatti Ramanathan Udhayasankar and Uthaman Danya., 2012. Phytochemistry and free radical scavenging activity of Wattakaka volubilis (Linn. f.) Benth ex. Hook f. (Asclepiadaceae) – A rare and threatened medicinal plant. Int. J. Pharm. Tech. Res., **4**(3): 1025-1032.

Basri D.F. and Fan S.H., 2005. The potential of aqueouse and acetone extracts of galls of Quercus infectoria as antibacterial agents. Indian J. Pharmaco., **37**:26–29.

Ciric A., Vinterhalter B., Vic K.S.F., Sokovic M. and Vinterhalter D., 2008. Chemical analysis and antimicrobial activity of methanol extracts of Celandine (Chelidonium majus L.) plants growing in nature and cultured in vitro. Arch. Biol. Sci. Belgrade., **60**(1):7–8.

David Beverly C. and Sudarsanam G., 2011. Ethnomedicinal plant knowledge and practice of people of Javadhu hills in Tamil Nadu. Asian J. Trop. Biomed., S79- S81.

Dayang F.B, Razinah S. and Paden M., 2005. Antimicrobial activities of ethanol and ethyl acetate extracts from the fruits of Solanum torvum. Malays. Appl. Biol., **34**(1): 31–36.

Gajendran K. and Ezhhilarasu A., 2012. Study of preliminary phytochemical analysis and antibacterial activity of selected medicinal plants (Gymnema sylvestre and Cocculus hirsutus). Int. J. Interdisci. Res. and Revs., **1**(1): 20-24.

Gamble J.S., 1935. The Flora of the presidency of Madras. Vol I-III. Adlard & Son, Ltd.; London.

Harborne L.B., 1973. Phytochemical methods, A guide to modern techniques of plant analysis. Chapman and Hall, London.

Naidu K.G., Naidu K.C.S. and Sujatha B., 2013. In vitro antibacterial and phytochemical analysis of leaves of Gymnema sylvestre Retz. R. Br. Int. J. Pharm Tech Res., **5**(3):1315-1320.

Mahajan D., Arjun R., Krishna K.M. and Gothandam, 2011. Ethnobotanical and Pharmacological study of Gymnema sylvestre, Pharmacologyonline, **3**: 785-798.

Mathew K.M., 1991. An Excursion Flora of Central Tamil Nadu: Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi.

Murugan M. and Mohan V. R., 2012. Phytochemical screening and antibacterial activity of *Gymnema sylvestre* (Retz) R. Br. Ex. Schultes and Morinda pubescens. J. Appl. Pharm. Sci., **2** (02): 73-76.

National Committee for Clinical Laboratory Standards Performance standards for antimicrobial disk susceptibility tests, 1993, Approved standard. NCCLS document M2-A5, Wayne, Pa: National Committee for Clinical Laboratory Standards.

Ndukwe I.G., Amupitan J.O., Isah Y. and Adegoke K.S., 2007. Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of Vitellaria Paradoxa. Afr. J. Biotech., **6**(16): 1905-1909.

Nikajoo L.T., 2009. Analgesic activity of aqueous and alcohol root extracts of Pergularia daemia (forsk.) Chiov. Int. J. Pharm. Pharm. Sci., **1**(1): 33-37.

Okwu D.E. and Okwu M.E., 2004. Chemical composition of Spondias mombin Linn. plant parts. J. Sustain Agric. Environ., **6**(2): 140-147.

Pritam J.S. and Sanjay B.B., 2011. Preliminary phytochemical screening of woody stem extracts of Abelmsochus Manihot and Wrightia tinctoria. Int. J. Pharmaceutica Sciencia., **1**: 59-6.

Karale P.A. and Karale M.A., 2017. A review on phytochemistry and pharmacological properties of milkweed family herbs (Asclepiadaceae). Asian Journal of Pharmaceutical and Clinical Research, **10**(11): 27-34.

Sripathi K. Shubashini and Uma Sankari, 2010. Ethnobotanical Documentation of a Few Medicinal Plants in the Agasthiayamalai Region of Tirunelveli District, India. Ethnobotanical Leaflets., **14**:173-81.

Upadhyay A., Upadhyaya I., Kollanoor-Johny A. and Venkitanarayanan K., 2014. Combating pathogenic microorganisms using plant-derived antimicro-bials: a mini review of the mechanistic basis. Biomed. Res. Int., Vol. 2014: 1-18.

Viji M. and Murugesan S., 2010. Phytochemical analysis and antibacterial activity of medicinal plant Cardiospermum halicacabum Linn. J. Phytol., **2**(1): 68–77.

Wani Minal, Faizan Ali Sarvar, Agarwal, Jyothi Despande, Siji Mathew and Madhukar Khetmalas, 2012. Qualitative phytochemical analysis and antimicrobial activity studies of Gymnema sylvestre R. Br. Acta Biologica Indica., **1**(1):121-124.

Warrier P.K., Nambiar V.P.K. and Ramankutty C., 1995. Indian Medicinal Plants. 1(5), Orient Longman Ltd., Madras.