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PHARMACOGNOSTICAL AND ANTIMICROBIAL SCREENING OF *GYMNEMA SYLVESTRE* R.BR, AND EVALUATION OF GURMAR HERBAL TOOTH PASTE AND POWDER, COMPOSED OF *GYMNEMA SYLVESTRE* R.BR, EXTRACTS IN DENTAL CARIES

***B.PARIMALA DEVI AND R.RAMASUBRAMANIARAJA**

SCBT, Department of Pharmacy, SASTRA University, Thanjavur

***Corresponding Author** parimaladevi2006@gmail.com

ABSTRACT:

The *medicinal plant Gymnema sylvestre* is famous for its antidiabetic potential in the herbal world is concerned. The Macroscopic, Physiochemical parameters, phytochemical screenings were carried out to facilitate quick identification and selection of the drug from various adulterants. The extracts prepared using successive solvent extraction techniques were screened for its antimicrobial activity by Agar well diffusion method against *Streptococcus mutans, Staphylococcus aureus*, *Streptococcus mitis* and *Candida albicans* with the doses 25, 50 and 100 mg/ml. The methanol extract showed strong antimicrobial activity with the zone of inhibition ranges from 12-23mm at 25mg/ml. The successive extracts of *Gymnema sylvestre* R.Br. was screened for its particle size, total microbial load, investigation with GC-MS and HPTLC studies. The *Gymnema sylvestre* hydro alcoholic extract and paste base, tooth powder base were used in the formulation of "Gurmar Herbal tooth paste" and "Gurmar Herbal Tooth powder" results found to be within the limits. This proves that the extract can be useful to treat the dental caries with the scientific documentation.

KEY WORDS: *Staphylococcus aureus*, *Streptococcus mitis*, *Candida albicans, Gymnema syvestre*

INTRODUCTION:

Dental caries is defined as indigenous infection caused by cariogenic bacteria. Different kinds of Gram-positive bacteria are closely related to the formation and progression of dental caries [(1)]. Organisms such as *Streptococcus mutans, Staphylococcus aureus, Streptococcus mitis,* these are primary cariogenic bacteria, and fungus like , *Candida albicans* from dental plaque by adhering to tooth surfaces through synthesis of extra cellular polysaccharides from sucrose. They subsequently metabolize sugar to organic acid such as lactic acid which is responsible for the demineralization of the tooth enamel [(2, 3, 4)]. The elimination of cariogenic bacteria from the oral cavity using antibacterial agents is one of the primary strategies for the prevention of dental caries. Extensive efforts have been made to find an active against dental caries. However an anticariogenic organism was found to be resistant to many of the antibacterial agent's viz., Penicillin, Chloramphenicol, Clindamycin, Ampicillin[(4, 5)]. In

addition they may lead to side effects including gastrointestinal problems[6].This drawback justifies further research and development of natural antimicrobial agents that are effective and safe for the host. It has been well documented that traditional medicinal plants considerable antimicrobial activity against various organisms[7] many plants were reported to inhibit the growth of many oral micro organisms [8]. Particularly *Streptococcus mutans, Staphylococcus aureus*, *Streptococcus mitis, Candida albicans* are control plaque and thus prevent caries have been investigated [9, 10]. In Present study we tried to explore Macroscopic, Physiochemical, phytochemical screening, Spectral Analysis like UV, HPTLC, GC-MS and Antimicrobial activity of various extracts of *Gymnema sylvestre, i*n dental caries.

MATERIALS AND METHODS:

Collection and Authentication of Plant materials:

The plant of Gymnema *sylvestre* R.Br,. was collected from Mooligai Pannai, 7km away from Thanjavur (Tamilnadu) in the month of August 2009. The plants was identified by local people of that village and authenticated by Dr. M. Jegadeesan, Professor and Head, Department of Environment and Herbal Science, Tamil University Thanjavur, The Voucher specimen (TUH 126A,) is preserved in our laboratory for future reference.

Chemicals

All the reagents used were of analytical grade obtained from S.D. fine chemicals Ltd, Mumbai.

Evaluation of Maroscopical and Microscopical Character:

The Macroscopic evaluation was carried out for shape, size, color, odor, taste and fracture of the drug.[11] Different physio-chemical values such as Ash value, extractive values, loss on drying, foreign organic matter, Crude fiber content, were determine. The microscopic studies were carried out using the method described by O Brien et al. [19]

Fluorescence analysis study of *Gymnema sylvestre* leaves powder:

Fluorescence analysis study of powdered drug material with different reagents was carried out to observe the color reactions [12]

Preparation of Successive Extract:

The leaves were dried under shade, powdered and passed through 40meshes and stored in closed vessel for further use. The dried powder material (500g) was subjected to soxhelt extraction with petroleum ether (40-60^{0}C), Chloroform, Methanol (in order of increasing polarity) for continuous hot extraction. The extracts were concentrated under reduced pressure to obtain the extracts solid residues. The percentage values (%w/w) were 7.7, 9.2, and 11.3 respectively.

Phytochemical Evaluation of three successive extracts:

All the three Successive extracts like Petroleum ether, Chloroform and Methanol extracts of *Gymnema sylvestre* were subjected to preliminary Phytochemical tests analyzed by the method of Peach, K and Tracy, M.V. et al [13]

Determination of Microbial load:

The plant material obtained was subjected to microbial analysis. 1ml of sample is taken and added to 9ml of sterile distilled water for preparing the serial dilution. The samples in the flask were kept in a mechanical shaker for few minutes to obtain uniform suspension of microorganisms. The dilution is 1:10 or 10^{-1}. From that 1ml of the 10^{-1} dilution is transferred to 9ml of sterilized distilled water. This is 1: 100 or 10^{-2}. This procedure was repeated up to 10^{-7} dilution. 0.1 ml of serially diluted samples was inoculated in to the sterile plate containing Nutrient agar, Salmonella Shigella Agar (SSA) and Potato Dextrose Agar (PDA) Medium by spread plate method. Nutrient agar,

and SSA plates were incubated at 37ºC for 24 hours and PDA plates were incubated at room temperature for 3-5 days. Bacterial and fungal colonies were counted using colony counter. *Salmonella, Shigella* and *E.coli* can be counted using SS Agar medium.

Antimicrobial screening of three successive extract of *Gymnema sylvestre*

Petroleum ether, Chloroform and Methanolic successive extracts of the plant like *Gymnema sylvestre* were used at various concentrations 100, 50 and 25 mg/ml respectively and tested against the microorganisms predominantly present in dental caries and infections associated.

Test Microorganisms

All the microbial strains of human pathogens used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. *Staphylococcus aureus (*MTCC 96), *Streptococcus mitis* (MTCC 2695), *Streptococcus mutans* (MTCC 890), and *Candida albicans* (MTCC 227) strains were used for the present study.

Selective antibiotics and media

Selective standard antibiotic drug (10µg/ml) was used against individual organism for Eg. Chloramphenicol against *S. aureus*, Clindamycin against *S. mitis*, Ampicillin against *S. mutans*, and *C. albicans* respectively. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used respectively for testing the antibacterial and antifungal activity

Determination of Antimicrobial Activity

Agar well-diffusion method [14] was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 h old - broth culture of respective bacteria and fungi. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37^0C for 18-24 hours for bacterial pathogens and 28°C for fungal pathogens. Respective proper controls of solvent plant extracts were also maintained. Diameter of the inhibition zones was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded for antimicrobial activity

Determination of particle size in Successive and macerated extract of *Gymnema sylvestre*

Particles sizing system (model 780 Accusizer) is standardized with distilled water before running the sample. With reference to this standard vale, the system is calibrated. A pinch of the sample is taken into the insoluble medium and mixed and stirred well. The medium is kept in a conical flask. It is taken in by the syringe and passed through the sensor. Few drops of the sample is added to the conical flask which is constantly stirred by the magnetic stirrer. The detector detects the number of particles per ml of the sample. The result derived is calibrated with reference to the baseline correction value. A data file can be retrieved to display the resulting particles size distribution with the desired.

UV spectral analysis of Successive extracts of *Gymnema sylvesre*

The successive extracts of *Gymnema sylvestre* analyzed for UV spectral studies using Perkin Elmer with the range 190 – 800nm

HPTLC Screening of *Gymnema* sy*lvestre*:

10g dried powdered leaves were extracted with 100ml of Ethanol for 6days maceration process. Extract were filtered to dryness on water bath. The dried mass left was weighed and redissolved in ethanol and analyzed by using CAMAG make HPTLC using the Mobile Phase Chloroform: Methanol: Glacial Acetic acid (5:1:1) and visualized by using Vanillin Sulphuric acid reagent at 105^0C for 5mins.

GC-MS investigation of successive extracts of *Gymnema sylvestre* R.Br.

A Volume of 1µl of clear extract was injected into GC-MS (PerkinElmer Clarus 500) with a oven programming of 50ºC @10 ºC/min to 150 ºC@8ºC/min to 280ºC (10min). The injector temperature was maintained at 280ºC. The split ratio was set as 1:8. The carrier gas used in the analysis was helium which had the flow rate of 1ml/min. A 30 m Capillary column of elite 5ms, with a Column id of 250 µm was used. The compounds were detected in the range of 40-450amu by matching with NIST library. [15],[16],[17]

Formulation of GURMAR Herbal Tooth paste :[18]

1.5gm of Gum tragacanth was soaked in specified quantity of water in one container. Then take 56.0gm of calcium carbonate, 2%w/w of *G.sylvestre* Hydro alcoholic extract, 1.0gm of Sodium lauryl sulphate in another container dry mixed. Add 22.0gm of glycerin to that and mix well until the mass gets slightly wet. Then gum tragacanth was added to it and wet completely, mixed well. The masses clumps are get mixed until all water molecules mix well. Then add 0.1gm of saccharine sodium and preservative like sodium benzoate sufficient quantity to it and mixed well to get thick paste. Finally add the peppermint oil of sufficient quantity.

Formulation of Gurmar Herbal Tooth powder:

Take 92.8gm of Calcium carbonate in one big container. To it add the *Gymnema sylvestre* (2%w/w) and mix thoroughly. To the Above mixed dry powder add 6.0gm of Sodium Lauryl sulphate and mix evenly. Then to the mixture add 0.2gm of powdered saccharin sodium to it and uniformly mix well. Then finally to the above mixture add flavor like peppermint quantity sufficient and mix completely and packed in well closed tight container.

Evaluation of Gurmar Herbal Tooth paste and Tooth powder evaluated for the properties like:

- **pH of the Product:** pH of the dispersion of 10% of the product in water is determined by pH meter.
- **Particle size:** Determined by Particle size analyzer. (model 780 Accusizer)
- **Volatile matters and moisture:** A specific amount of the product required to be taken in a dish and drying is to be done till constant weight. Loss of weight will indicate percentage of moisture and volatile matters.
- **Cleansing property:** This is studied by measuring the changes in the teeth were brushed for 2 weeks and condition of teeth was assessed before and after use.
- **Foaming character:** This test is specially required for foam forming tooth pastes or tooth powders. Specific amount of product can be mixed with specific amount of water to be shaken. The foam thus formed is studied for its nature, stability, washability.
- **Flow property:**Flow property is determined by Angle or repose in open ended and Funnel method. ($\theta = Tan^{-1}$ h/r)
- **Bulk density** Bulk density is determined by Tapped and untapped volume of the powder.

RESULTS AND DISCUSSIONS:

Macroscopic characters

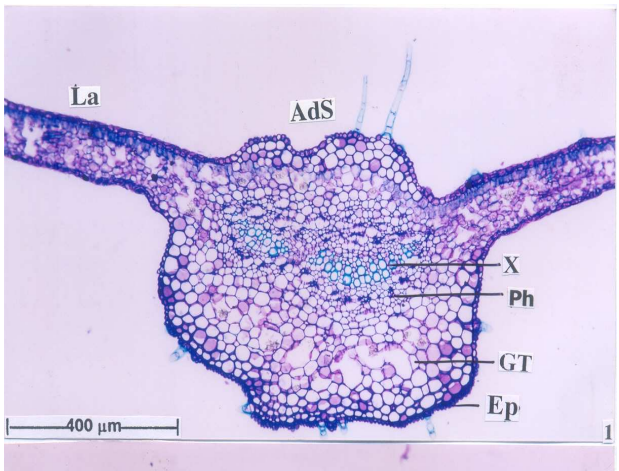
The following macroscopic features were observed for the leaves; the leaves were Green in colour and were 2.2-2.0cm in length and 1.3-1.30cm in width. Their shape was even-pinnate or ovate, the taste was extremely bitter, and had a Characteristic odour. It had pubescence. The leaves were simple, opposite with acute apex, rounded to cordate base and had a reticulate venation and had a 5-13cm long pubescent petiole.

Microscopy:

Leaf is dorsiventral, mesomorphic, hypostomatic even and fairly thin. Midrib is prominent, broadly hemispherical on the abaxial side and shortly raised into broad hump on the adaxial side (Fig 1.1). Ground tissue of the midrib consists of outer narrow zone of collenchyma, rest of the cells being compact, thin walled parenchymatous. Vascular tissue consists of broad, prominent and arc of radial multiples of xylem elements with phloem nests both on the upper and lower sides of the xylem strands [Bicollateral Vascular strand- (Fig 1.1.).

The lamina has squarish adaxial cells with thin cuticle and convex inner tangential wall. Abaxial epidermis is also similar to adaxial epidermis; but the cells are smaller [fig -1.2]. Palisade mesophyll cells are long, narrow, single layered and occupied one third of the thickness of the lamina. The spongy parenchyma cells are lobed and loosely arranged. The vascular bundle of the lateral vein consists of a small, but prominent collateral vascular strand with this bundle sheath [Fig 1.2]. Large calcium oxalate crystals are fairly abundant in the midrib ground tissue [Fig 2.1] and mesophyll tissue [fig 2.2].

Fig 1.1



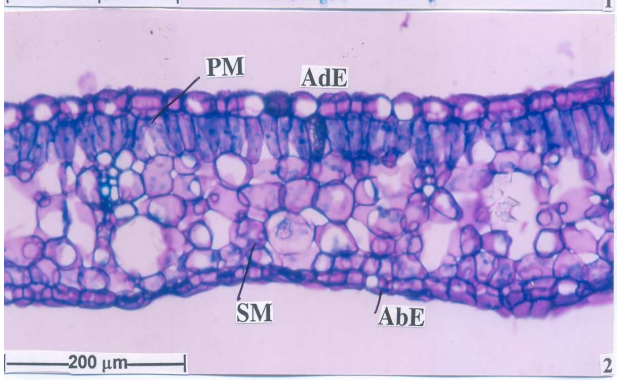
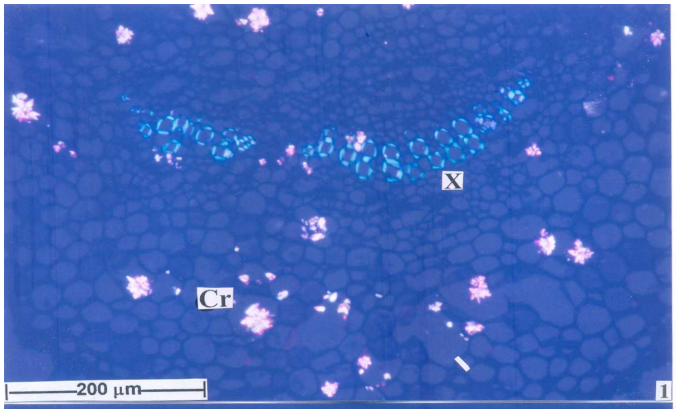


Fig 1.2

Fig 2.1



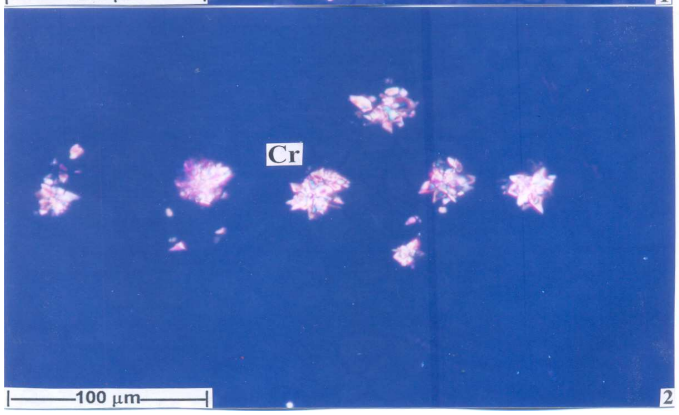


Fig 2.2

Abbreviations:

[AbE-Abaxial epidermis; AdE-Adaxial epidermis; Ads-Adaxial side; Ep-Epidermis; GT-Ground tissue; La-Lamina; ph-Phloem; PM-Palisase messophyll; SM-Spongy mesophyll; X-xylem Cr-crystal]

Physiochemical Parameters

Based on the polarity the extractive values of the leaves of were analyzed. The extractive value was highest in water and was recorded to be 30.56%w/w, and methanol soluble extractive value was about 20.8%w/w .The different ash values and the different physiochemical parameters were screened and are presented in the table No.1.

Table No: 1

Physiochemical Parameters of *Gymnema sylvestre* R.Br. leaf powder

S.No	Parameters	%w/w	S.No	Parameters	%w/w
1.	Hexane soluble extractive	3.04	8.	Water soluble extractive	30.56
2.	Pet ether soluble extractive	5.24	9.	Foreign organic matter	0.38
3.	Chloroform soluble extractive	2.344	10.	Loss on drying	5.6
4.	Acetone soluble extractive	7.12	11.	Crude fiber content	14.05
5.	Ethanol soluble extractive	9.256	12.	Total ash	8.3
6.	Ethyl acetate soluble extractive	3.92	12.	Acid insoluble ash	0.9
7.	Methanol soluble extractive	20.8	13.	Sulphated ash	15.3

Table No: 2

Fluorescence analysis study of *G.sylvestre* leaves powder

Sample	Color in day light	Color in 366nm
Powder	Green	Brownish green
Powder + Sodium hydroxide in methanol	Brownish yellow	orange
Powder+Sodium hydroxide in water	Brownish yellow	Green
Powder+1N Hydrochloric acid	Light brown	Reddish brown
Powder+50%Nitric acid	Reddish brown	Light green
Powder + 50%Sulphuric acid	Light brown	purple

Table No: 3

Preliminary phytochemical screening of three successive extract of *Gymnema sylvestre*

Phytoconstituents	Pet ether	Chloroform	Methanol
Alkaloids	-	-	-
Anthaquinones	-	-	-
Carbohydrates	+	-	+
Flavonoids	+	+	+
Phenolic groups	+	+	+
Saponins	-	-	+
Steroids	-	-	-
Tannins	-	-	+
Tritepenes	+	+	+

+ = Present - = Absent

Successive extracts of *G.sylvestre*, were qualitatively screened and are presented in the table No: 8. Triterpenes, Phenolic groups and flavonoids were present in all the three extracts, Carbohydrates;

Saponins were present only in the Pet ether and methanolic extract of *G.sylvestre*, Tannins and Saponins mainly present in the Methanolic extract of *G.sylvestre*.

Table No: 4

Total Microbial Count of different extracts of *Gymnema sylvestre*

S.No	Bacterial Name	Pet ether	Chloroform	Methanolic	Ethanolic	WHO Limit	Inference
		Cells in Sample/g					
1.	*E.coli*	Nil	Nil	Nil	Nil	10^2	Within Limit
2.	*Salmonella sp.*	Nil	Nil	Nil	Nil	Absence	Passes the test
3.	*Shigella sp.*	Nil	Nil	Nil	Nil	Absence	Passes the test
4.	Total Heterotrophic Bacteria	6×10^4	Nil	100×10^4	2×10^3	10^7	Within Limit
5.	Yeast &Mould	Nil	1×10^3	20×10^2	3×10^2	10^4	Within Limit

Table: 5

Anti Microbial activity of Successive extracts of *Gymnema sylvestre*:

Micro organisms	Zone of Inhibition											
	Pet ether mg/ml			Std 10µl/ml	Chloroform mg/ml			Std 10µl/ml	Methanol mg/ml			Std 10µl/ml
	100	50	25		100	50	25		100	50	25	
***S.aureus* MTCC96**	-	-	-	34 (Ch)	-	-	-	32 (Ch)	18	15	14	36(Ch)
***S. mitis* MTCC 2695**	23	20	16	33 (Cl)	17	14	12	36 (Cl)	21	19	13	38(Cl)
***S. mutansi* MTCC 890**	22	16	15	36 (A)	-	-	-	33 (A)	27	25	23	33 (A)
***C.albicans* MTCC 227**	-	-	-	32 (P)	-	-	-	31 (P)	16	16	12	32 (P)

Ch – Chloramphenicol; Cl- Clindamycin; A – Ampicillin; P – Penicillin,

Std- Standard drug

Successive Extracts of *G.sylvestre* were Antimicrobial activity screened and are presented in the table No: 9. from the results, Methanolic extracts of G.sylvestre having marked activity against the microorganisms tested in dose dependent manner. In addition the phytoconstituents present in the extracts may enhance the activity that can be justified if the study can be conducted with the plant components.

Particle size analysis of Successive extracts of *Gymnema sylvestre*:

1. **(*Gymnema sylvestre*) Pet Ether extract:** The Particle size of the given sample ranges from **0.57** to **27.39micron** and the average particle number range is 2.43.

2. **(*Gymnema sylvestre*) chloroform extract:**

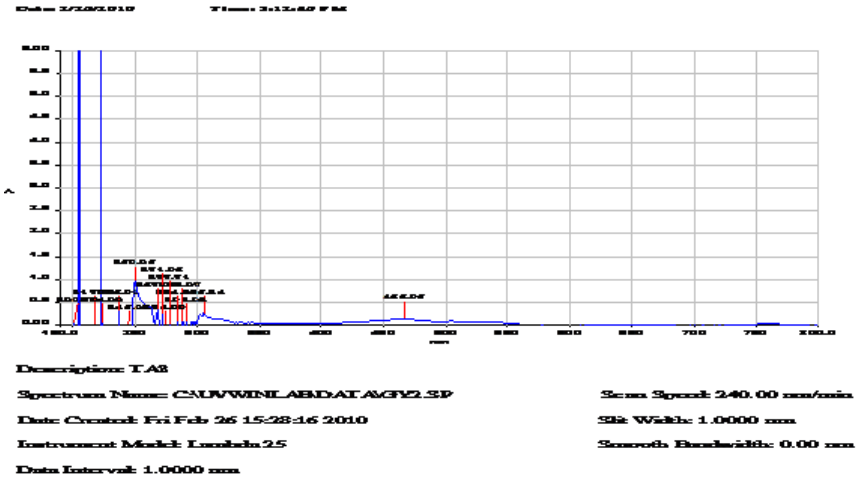
The Particle size of the given sample ranges from **0.57** to **27.39micron** and the average particle number range is 4.29.

3. (*Gymnema sylvestre*) Methanol extract: The Particle size of the given sample ranges from **0.52** to **314.10micron** and the average particle number range is 16.25.

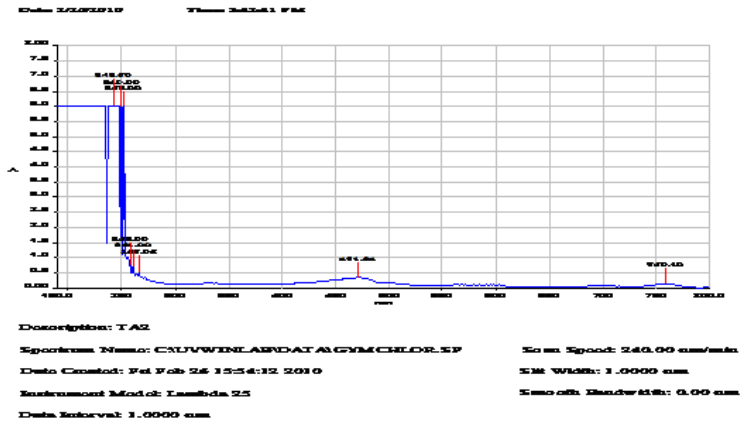
UV Scanning of Successive and Macerated Extracts of *Gymnema sylvestre*:

Plot No: 1

Sample Name*: Gymnema sylvestre* (Pet ether Extract)



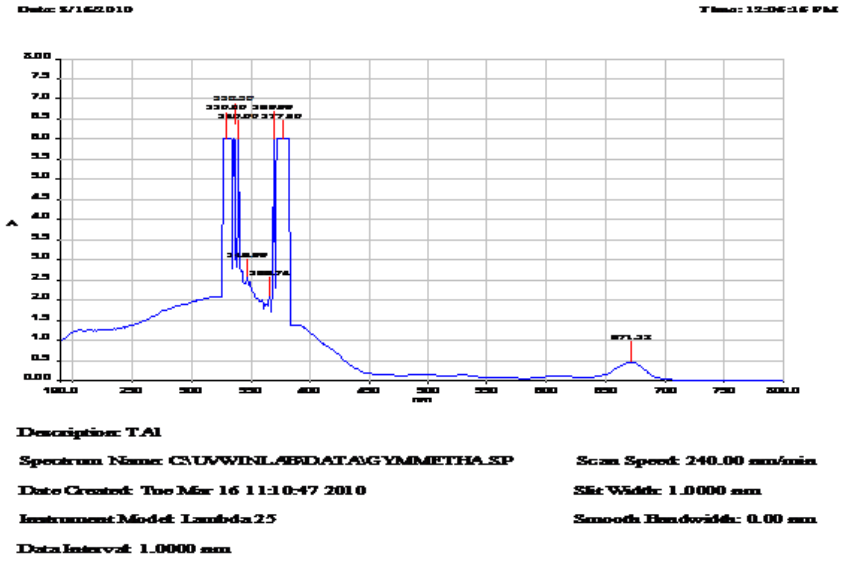
Plot No: 2



Sample Name*: Gymnema sylvestre* (Chloroform Extract)

Plot No: 3

Sample Name*: Gymnema sylvestre* (Methanol Extract)



As Qualitative Analysis the Spectral maxima for *Gymnema sylvestre* of various extract like Pet ether, Chloroform, Ethanol and Methanol were screened for Preliminary Phytochemical Constitution.

Table-6

Particle size analysis of Extracts

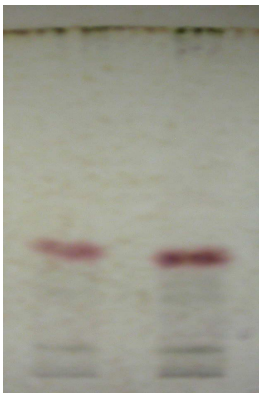
S.No	Extract	λmax	Absorbance
1.	Pet ether	250	0.9658
2.	Chloroform	250	6.0015
3.	Methanol	370	6.0012

From the Above result obtained, the absorbance was found to be of about 6.0012 at Maximum wavelength of 370nm. This infers that most of the secondary metabolites were found to be in Methanolic extracts. Since the Most of the phenolic secondary metabolite particular flavonols lies over the range of 340-380nm. The results indicate that the secondary metabolites particularly flavonols may be present were identified.

HPTLC Screening of Ethanolic extract of *Gymnema sylvestre*:

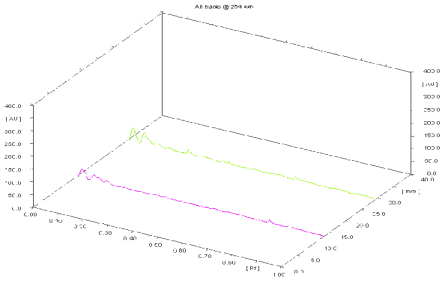
Fingerprinting

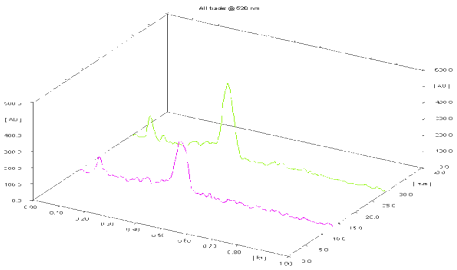
@254nm **Spray**



3D DISPLAY

254nm **SPRAY**

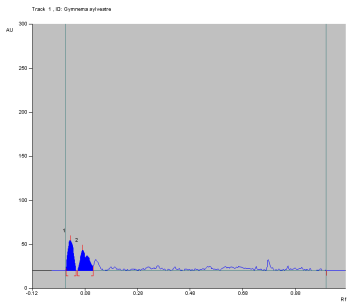


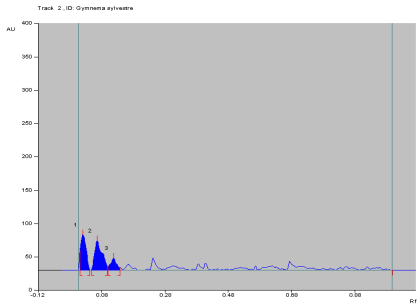


PEAK DISPLAY

@ 254nm

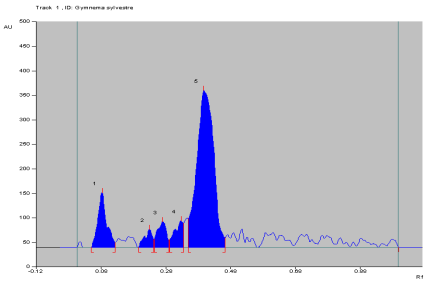
Plot-4 TRACK 1 **Plot-5 TRACK 2**



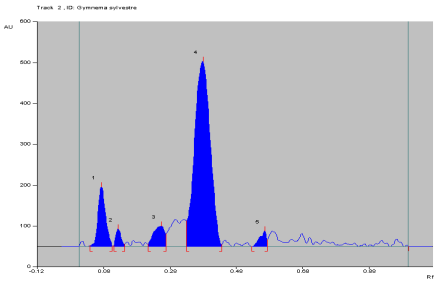


Spray

Plot-6 TRACK 1



Plot-7 TRACK 2



TABLES-7

@ 254nm

TRACK 1

Peak	Rf Value	Area (AU)
1.	0.03	560.2AU
2.	0.07	538.9AU

Track 2

Peak	Rf value	Area (AU)
1	0.02	701.8 AU
2	0.07	815.8 AU
3	0.12	265.8 AU

Tables-8

Spray

Track 1

Peak	Rf value	Area (AU)
1	0.09	2351.2 AU
2	0.23	681.7 AU
3	0.27	1124.6 AU
4	0.33	1100.8 AU
5	0.40	13004.7 AU

Track2

Peak	Rf value	Area (AU)
1	0.07	2555.8 AU
2	0.13	521.6 AU
3	0.26	1325.4 AU
4	0.38	16426.3 AU
5	0.57	567.0 AU

GC-MS investigation of three successive extracts of *Gymnema sylvestre* R.Br.

The results of the GC-MS analyses on Successive extracts like Pet ether, chloroform and methanolic extracts of the leaves of *Gymnema sylvestre* is presented in Tables.

A total of 19 compounds were identified from the Pet ether extracts of *G.sylvestre.* The identified compounds were of different types of Terpenes, and the highest % Peak area of 69.136 is limonene (Retention time 8.47), Saturated and unsaturated fatty acids, recorded the next highest % peak area of 14.867. Hexadecanoic acid ethyl ester(Retention time 23.11), Phenolic groups had a % Peak area of 1.175 and the name of the phenolic group was identified as Phenol, 2,4-bis(1,1-dimethylethyl)- (Retention time 16.42), Hydrocarbons followed the order of % peak area

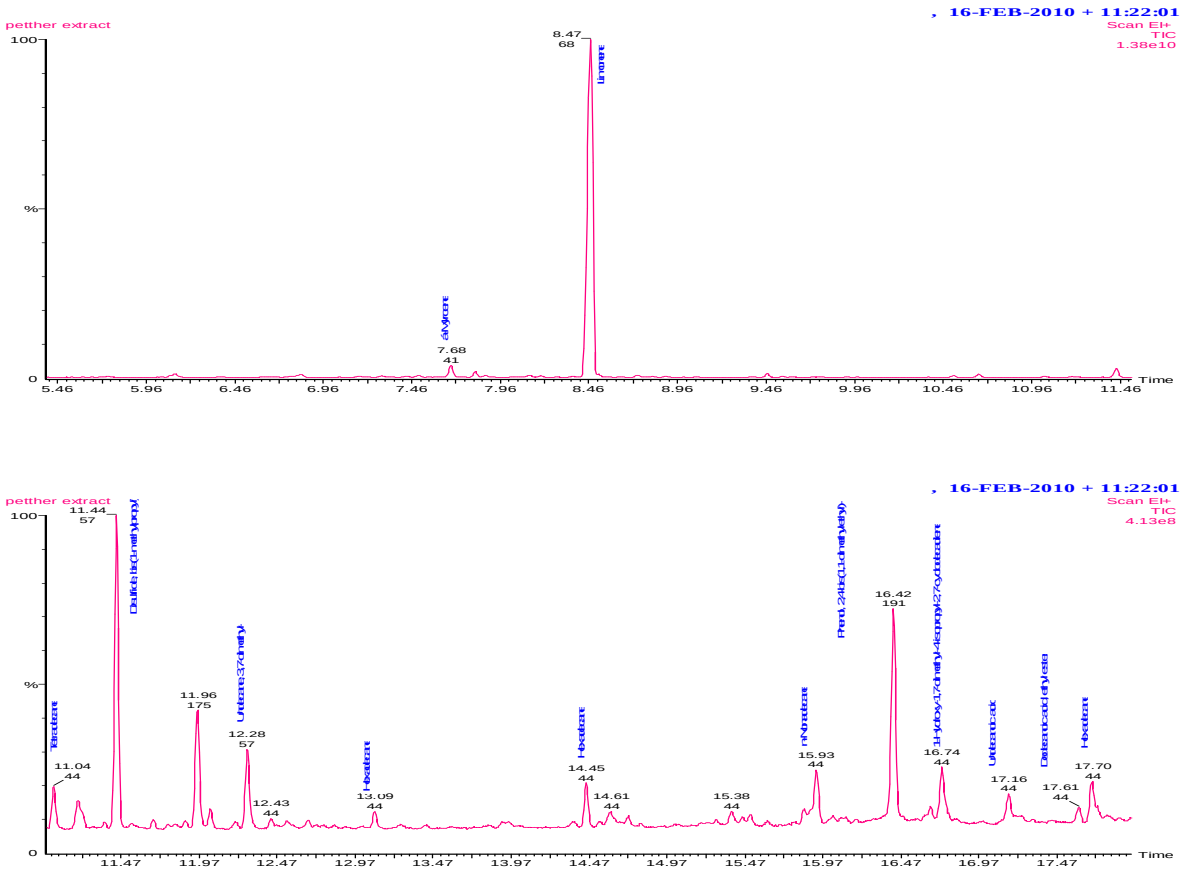
of 0.414 and the name was identified as Undecane, 3,7-dimethyl-(Retention time 12.28).

A total of 13 compounds were identified from the Chloroform extracts of *G.sylvestre*. The identified compounds were Single Alkaloids and recorded a % peak area of 0.186 whose name has been identified as 2-Isopropylpiperazine (Retention time 3.96), hydrocarbons ranked next with a% Peak area of 1.957 and the peak had been identified as Tridecane (Retention time 8.74). Aldehyde groups followed the list with a % peak area of 0.125 and its name had been noted as Butanal, 3-hydroxy- (Retention time 9.56), Saturated and unsaturated fatty acids followed the list with a % Peak area of 16.068 and the corresponding name has been recorded as 9-Octadecenoic acid (Z)-, methyl ester (Retention time is 24.49).

A Total of 7 compounds had been identified from the Methanolic extract of *G.sylvestre*. The identified compounds include Terpenes recording the highest % Peak area of 52.101 and the respective name has been identified as Limonene (Retention time 8.50), Saturated and Unsaturated fatty acids with a % Peak area of 0.787 have recorded the name as 9-Octadecenoic acid (Z)-, methyl ester (Retention time 24.44).

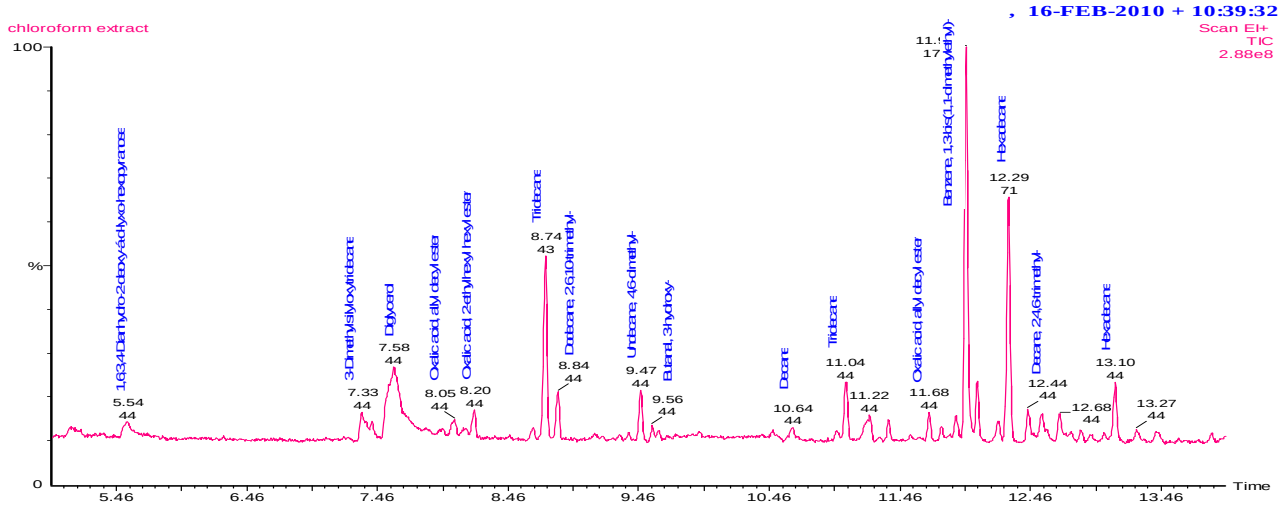
Plot No: 8

Chromatogram of Pet ether Extract of *Gymnema sylvestre*:



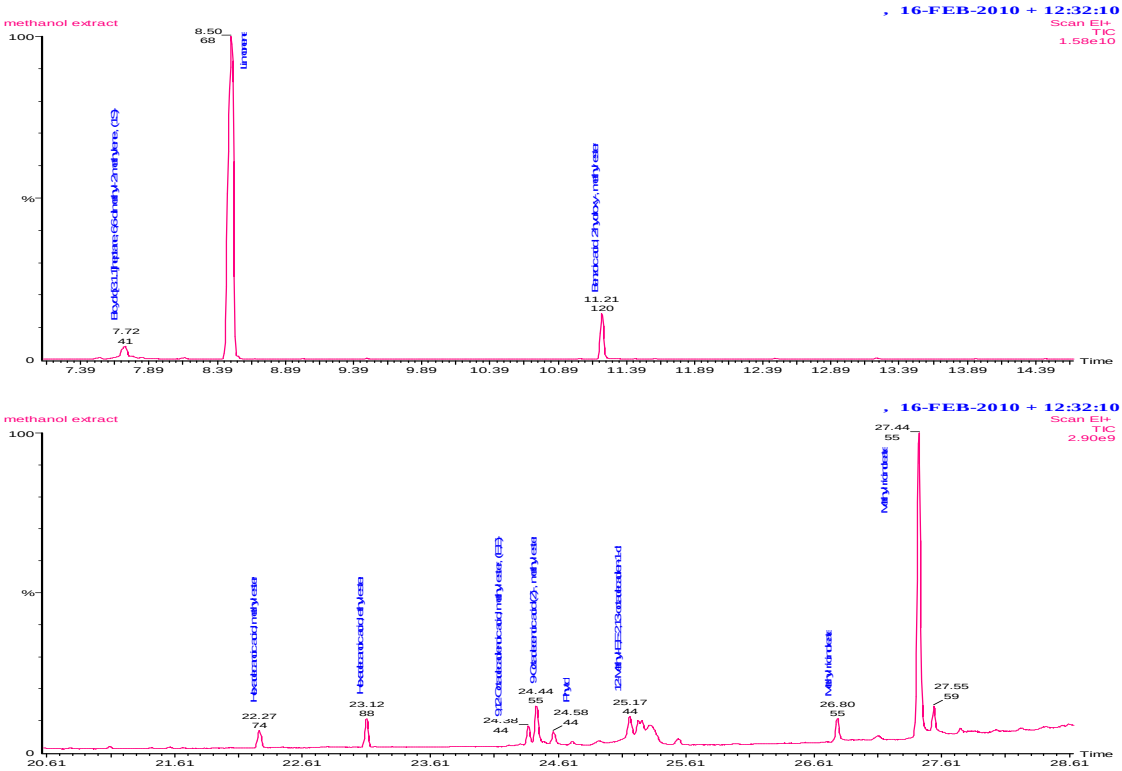
Plot No: 9

Chromatogram of Chloroform Extract:



Plot No: 10

Chromatogram of Methanolic extract of G.sylvestre:



Evaluation of GURMAR Herbal Tooth paste

- pH - 9.25

- Particle Size **-** The Particle size of the given sample ranges from 0.57 to 27.39micron and the average particle number range is **3.86**.
- The percentage of Volatile matter is **13.5%**w/w
- Cleansing property before and after use

- **Foaming character**

Table N0: 19

S.No	Percentage of Solution	Volume of foam (ml)
1.	1%	3.4
2.	2%	3.4
3.	5%	3.2

Evaluation of Gurmar Herbal Tooth powder:

- pH - 10.07
- Cleansing property (After and before use)

Table No: 19
Flow properties determined by angle of repose

S.No	Method	Radius	Height	h/r	$\theta = Tan^{-1}$ h/r
1.	Open ended Method	4.25cm	4.5cm	1.0588	46.64
2.	Funnel method	4.1cm	3.8cm	0.9268	46.64

- The Percentage of **Volatile matter** is 10.5%w/w
- **Bulk density:**
  a. Untapped - 0.3783gm/ml
  b. Tapped - 0.5447gm/ml

Conclusions: Dental caries are the most common oral infectious disease among children and old age. The prevention strategy against dental caries includes the elimination of cariogenic micro organisms from the oral cavity, inhibition of their plaque formation and the enhancement of tooth resistance to demineralization. In the former strategies, phytochemicals have been widely studied for their antimicrobial activity. A variety of plants with potent activity are known to be traditionally used for dental hygiene world-wide . Antibiotics and other antimicrobial agents are effective in the prevention and treatment of dental caries, but they also cause undesirable side effects such as ecologic disturbance of oral and gut flora. Furthermore, viridans group streptococci including *S, mitis, S.mutans*, *C.albicans* most representative human cariogenic bacteria are moderately resistant to antibiotics Therefore, search for the antimicrobial herbs could offer an effective alternative to antibiotic strategies for oral infection disease like dental caries. Among the Three marketed hydro alcoholic extracts G*ymnema sylvestre*, *Mentha arvensis, Solanum suraatense* effective against the Micro organisms like caries produce bacteria and fungi *Gymnema sylvestre* R.Br. showed highest activity at minimum concentration. The plant *Gymnema sylvestre* was screened for its Physiochemical parameter, Florescence analysis, Qualitative and Total microbial load showed that they all within limit. Extraction was carried out in the order of polarity. Among the three Successive extracts effective against list out the Micro organisms like *S. aureus, S. mitis, S. mutansi, C.albicans* Methanolic extract of *Gymnema sylvestre* showed highest activity at minimum concentration. Thus from our findings, it was concluded that the bioactive principles present in the extracts may be responsible in the treatment of dental caries. The UV scanning to identified highly present flavonols groups in all the extracts. Different Rf values are identified 254 nm and spray reagent techniques by HPTLC method of Macerated Ethanolic extract. Future analysis purposed well knowledge to elaborately identified GC-MS investigation of Successive extracts. Different Rf values are identified 254 nm and spray reagent techniques by HPTLC method of Ethanolic extract. Developing countries like India having the percentage of poor people more, to meet with the demand of the poor public, the paste like Gurmar may serve the purpose once the evaluation and detailed studies may over.

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