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SCREENING OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT *GYMNEMA SYLVESTRE* (ASCLEPIADACEAE)

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ABSTRACT:

To evaluate antimicrobial activities of aqueous from, Acetone, Petroleum ether, Methanol, D/W, Dimethylformamide, Dimethyl Sulfoxide extract of Plant *Gymnema sylvestre* (*G. sylvestre*) leaves and Steam.

The antimicrobial screening of extract *G. sylvestre* against common microbes like *Bacillus cereus (B. cereus), Escherichia coli (E. coli),* by agar well diffusion method, where MIC, MBC, MFC was carried out. The aqueous and Methanol leaf and steam extract showed significant antibacterial activities against the selected microorganisms and were compared to the standard antibiotic respectively.

The leaves of *G. sylvestre* might represent a new antimicrobial source with stable, biologically active components that can show a scientific base for the use in modern medicine.

KEYWORDS: *Gymnema sylvestre, Bacterial strains, Disc-diffusion method, Antibacterial assay, Phytochemical.*

INTRODUCTION:

Plants have been used as medicinal purposes in the ancient period. Population rise, an insufficient supply of drugs and the prohibitive cost

of treatments, side effects of several synthetic drugs and development of resistance, now used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Medicinal plants have been used for a long time as medicines for human ailments because they contain some therapeutic compounds for potential (Jamine *et. al;* 2007). The neuropathic treatment for diseases has been explored extensively since ancient times and gaining momentum in the present scenario. Indian flora accounts for about 45,000 plant species out of which several thousand have pharmacological significance (Grover *et. al;* 2002). During the last few years, an increase in the study of medicinal plants and their traditional use in different parts of the world (Lev, 2006). Most of the plants have shown varying degrees of hypoglycaemic and anti-hyperglycemic activity (Grover *et. al;* 2002). Thus over 50% of these current tablets are of natural produce substance and as such play a vital role in drug improvement in the pharmaceutical industry (Jeyachandran and Mahesh, 2007). The bioactive constituents discovered in many plant species are isolated for direct use as drugs, lead compounds, or pharmacological agents. These normal strategies might provide a natural key to release diabetic issues (Babu *et. al;* 2006). The chemical constructions of a Phyto molecule play an indispensable function in its antidiabetic activity. Several plant species being an essential source of terpenoids, flavonoids, phenolics, coumarins, and other bioactive parts have shown discounts in blood glucose levels (Zhang *et. al;* 2009).

Apart from resistance, some antibiotics have serious adverse aspect effects which restriction their applications, so there is pressing want to advance new antimicrobial dealers that are very high-quality with minimal facet effects, and characterize a conceivable supply of novel antibiotic prototypes (Maureer *et. al;* 1996). Medicinal plants and herbal products have been used to treat a range of human health troubles and there has been renewed activity in their use for built-in cancer management. (Kamble and Gacche, 2019). *Gymnema* is a famous herbal medicinal drug used worldwide.

The leaves of *G. sylvestre* have been used in India for the cure of diabetes. Various antidiabetic plant extracts like *Acacia arabica, Aegle marmelos, Allium cepa, Allium sativum, Aloe vera, Azardirachta indica, Brassica juncea, Cajanus cajan, Capparis decidua, Citrullus colocynthis, Coccinia indica, Hibiscus rosa-sinesis, Ipomoea batatas, Mangifera indica, Momordica charantia, Musa sapientum, Nelumbo nucifera, Punica granatum, Syzigium cumini, Trigonella foenum graceum, Vinca rosea* and formulations like those of chromium have been used and clinically tested for their activity as nicely as possible facet effect (Makwana *et. al;*., 2016, Shane 2009). The phytochemicals in leaf extract had been additionally analyzed via fuel chromatography coupled to mass spectrometry and recognized for the presence of terpenoids, glycosides, saturated

and unsaturated fatty acids, and alkaloids in three exclusive leaves extract, namely, petroleum ether, chloroform, and methanol as solvents used for extraction (Sathya et. al;, 2010). The existing evaluation is research replace on *Gymnema sylvestre*, a rare herb with widespread medicinal attributes with an overview of its ethnobotanical uses, phytochemistry dealing with an in-depth study of its phytochemicals, and their bioactivities.

The leaves *of G. sylvestre* have been used in India for the remedy of diabetes. The plant has antidiabetic potential, hypolipidaemic and two antiatherosclerotic, insulinotropic anti-inflammation, anticancer cytotoxic, anti-oxidant, wound healing, leishmanicidal and antimicrobial activities (Baskaran et. al; 1990, Ramachandran and Natarajan, 2010). The reason for this work is the challenge with the screening of the plant for in vitro antibacterial houses of *Gymnema sylvestre* was examined in opposition to the bacterial strains antibiotic.

MATERIALS AND METHODS:

Collection of plant materials:

The *G. sylvestre* leaves and stem collected during June-July of 2019 in and around North Gujarat, Patan, and authenticated by the Department of Botany. The voucher specimens were kept in the Department of Botany in M.N. Science College, Patan, India.

Extraction procedure

Dark dried leaves and stem (200g) were coarsely powdered and subjected to successive solvent extraction by Continuous hot extraction (Soxhlet). The extraction was accomplished with different solvents in their growing order of polarity such as Aqueous, Acetone, Petroleum ether, Methanol, D/W, Dimethylformamide, Dimethyl Sulfoxide. Each time the glassware was air-dried and later extracted with different solvents. All the extracts were focused on distilling the solvent in a rotary flash evaporator as a bioactive material.

Test organisms

The microorganisms used for the test were *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), and cultures used in the study were obtained from Culture depositary members from the Department of Life Sciences, Hemchandracharya North Gujarat University, Patan.

Culture media and inoculums preparation

Nutrient agar/ Rose Bangal agar (Himedia, India.) was used as the media for the culturing of strains. Loops full of all the microbial cultures were inoculated in the nutrient broth at 37 °C for 72 hrs.

Antimicrobial activity study

Antimicrobial Activity of the *G. sylvestre* leaves and Steam extracts viz. aqueous, Acetone, Petroleum ether, Methanol, D/W, Dimethylformamide, Dimethyl Sulfoxide was determined, using

with agar well diffusion approach. Approximately 20 mL of molten and cooled media was poured in sterilized Petri dishes. The plates have been left kept at room temperature to check for any activity to appear. The test organisms were grown in broth for 24 h. in 100 ml broth subculture of each test organism ($1x10^{-5}$ cfu/mL) was used to prepare the lawn. Agar wells of 5 mm diameter bore were prepared with the assist of a sterilized stainless metal cork borer. Five wells had been organized in the agar plates. The wells were labeled as A, B, C, D and E revered to Antibiotic, Original extract,1:5 Dilution,1:10 Dilution, Solvent 'A' properly was loaded with 10µL of Antibiotic leaves extracts, 'B' properly used to be loaded with 10 µL of Original extract leaves extracts, 'C' well was loaded with 10 µL of 1:5 Dilution leaves extracts, 'D' nicely was once loaded with 10 dilution leaves extracts and 'E' well used to be loaded with Solvent. Two various bactericides were used as ideal controls. The plates containing the organisms and extracts were incubated at 37°C. The plates were examined for observation of zones of inhibition, which show up as a clear zone around the wells. The diameter of such zones of inhibition was once measured using a meter ruler and the suggested cost for every organism was recorded and expressed in millimetre for leaves and steam.

Antifungal activity study.

The Agar well diffusion method is utilized to test the antibacterial action of extricate. The agar sort salt concentration, hatching temperature, and atomic estimate of the antimicrobial component impact come about gotten with agar dissemination test. The foremost broadly utilized elective strategy in the common microbial measure is the serial weakening of the extricate in several test life forms to decide for MIC. The agar well dissemination strategy, plates were hatched for 48 hours at 400c at that point the restraint one was watched, distinctive the test life form culture from the *A.niger*, *Chaetomium,* and *Neurospora*. With leaf and Steam (David and Sudarsanam, 2013).

RESULTS AND DISCUSSION:

Phytochemical screening of *Gymnema sylvestre* :

The preliminary phytochemical study of the methanol extract of leaf and stem of *Gymnema sylvestre* exposed to the group of presence was shown in a table : 1. Based on the colour reactions and precipitations, the possibility of the isolable compounds in the Methanolic extract of *Gymnema sylvestre* plant leaf and stem are phenolic compounds, alkaloids, flavonoids, sterols, proteins, tannins, saponin, glycosides, and triterpenoid. That leaves and stem offer a wider array of phytochemicals. Phytochemical tests of *Gymnema sylvestre* in Anthraquinones, Phenol, Steroids, Tannins, were given positive results in both leaf and stems.

Antimicrobial activity

The antimicrobial activity of leaf and stem extracts viz. Aqueous, Acetone, Petroleum ether, Methanol, D/W, Dimethylformamide, Dimethyl Sulfoxide, were evaluated against two test organisms concerning antibiotic (Table 1), the leaf extracts of *G. sylvestre*, i.e. Aqueous, Acetone, Petroleum ether(P.E), Methanol, D/W, Dimethylformamide (D.M.F), Dimethyl Sulfoxide (D.M.S.O). Applied combination with antibiotic of test strains was in case of leaf extract (E.coli + Streptomycin), Acetone and Methanol showed significant antimicrobial activity against similarly concerning another extract. Similarly in case of stem DMSO express 72 mm , concerning D.M.F. 72 mm, D/W 58 mm, Acetone 58 mm, Methanol 53mm and in P.E 42mm observe. In the second stage the leaf extracts of *G. sylvestre*, i.e. Aqueous, Acetone, Petroleum ether, Methanol, D/W, Dimethylformamide, Dimethyl Sulfoxide. Applied combination with antibiotic of test strains.were in case of leaf extract (*Bacillus subtilius + Panicillin)* were D/W 54mm, PE 39 express dominant as respet othern solvents, in case of stem D.M.F. 82mm, DMSO 45, D/W 43 represent respectively.

Antifungal activity

The antifungal activity of leaf and stem extract of *Gymnema sylvestre* is shown in table no. 5. Methanol, Acetone, and D.M.S.O extract were showed a zone of inhibition against *A.niger*, *Neurospora*, and *Chaetomium*. DMSO and Methanol leaf extract and Acetone stem extract showed higher activity against *A.niger, Neurospora,* and *Chaetomium.* As these solvent used in new drug discovery, it was plant-based, it is safe for human consumption.

FTIR :

The crude extract of *Gymnema sylvestre* is analyzed by the FT-IR spectra to find out the functional groups. Fig 1 shows the FT –IR spectra of crude extract of *Gymnema sylvestre*. The observed stretching frequencies of crude extract of *Gymnema sylvestre* stem shows bands are 3334, 2917, 1801, 2899, 1718, 1825, 1735, 3344, and 1718. Alkane, Alkynes, Ester, Ketones, Carbonyl, and Alkene groups were present in the stem of *Gymnema sylvestre.*

CONCLUSION:

Present day's ethnobotanical employments of characteristic compounds, particularly of plant beginning gotten much consideration as they are well-tried for their adequacy and by and large accepted to be secure for human utilize. The Methanol and Petroleum ether extricates of *Gymnema sylvestre* have shown great antimicrobial action against both gram-positive and gram-negative microorganisms and Methanol, Acetone, and DMSO has appeared great antifungal movement showing its potential within the advancement of modern phytopharmaceuticals. As these drugs are

plant-based, they can be considered secure for human consumption. In conventional improvements to remedy diabetes, *Gymnema sylvestre*, has a vital put among such antidiabetic therapeutic plants. Since it has recovering capacity of β-cells, and thus it may be utilized as a sedate for treating diabetes mellitus.

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Table 1: Screening of Phytochemical

Phytochemical screening							
S. No	Chemical test	Leaf	Stem	S. No	Chemical test	Leaf	Stem
1	Alkaloids	+ve	-ve	8	Terpenoids	+ve	-ve
2	Anthraquinones	+ve	+ve	9	Xanthoprotien	+ve	-ve
3	Flavonoids	-ve	-ve	10	Sugar	+ve	ve
4	Phenol	+ve	+ve	10.1	Molish Test	-ve	-ve
5	Saponins	+ve	-ve	10.2	Fehling test	-ve	-ve
6	Steroids	+ve	+ve	10.3	Bendicts Test	+ve	-ve
7	Tannins	+ve	+ve	11	Phlobatannins	-ve	-ve

Table 2: Antimicrobial activity of *Gymnema sylvestre* (E.coli + Streptomycin)

Antimicrobial activity of *Gymnema sylvestre* (*E.coli + Streptomycin*)						
Name of the extract	**Plant part**	**Zone of inhibition (mm)**				
		Antibiotic	**Original extract**	**1:5 Dilution**	**1:10 Dilution**	**Solvent**
Methanol	**L**	57	47	0	0	0
	S	53	47	0	0	0
D/W	**L**	57	13	0	0	0
	S	58	18	0	0	34
D.M.F.	**L**	9	31	0	0	0
	S	72	26	23	22	25
DMSO	**L**	12	0	0	0	0
	S	75	33	23	18	12
Acetone	**L**	47	56	34	0	25
	S	58	55	15	0	19
P.E	**L**	50	46	0	0	0
	S	42	38	0	0	0

Table 3: Antimicrobial activity of Gymnema sylvestre (E.coli + Panicillin)

Antimicrobial activity of *Gymnema sylvestre* (*E.coli* + *Panicillin*)						
Name of the extract	**Plant part**	**Zone of inhibition (mm)**				
		Antibiotic	**Original extract**	**1:5 Dilution**	**1:10 Dilution**	**Solvent**
Methanol	**L**	29	54	0	0	0
	S	29	48	0	0	12
D/W	**L**	54	9	0	0	0
	S	43	14	0	0	0
D.M.F.	**L**	25	43	0	0	0
	S	82	33	0	0	26
DMSO	**L**	12	0	0	0	0
	S	45	0	0	0	0
Acetone	**L**	18	0	0	0	12
	S	14	19	0	0	0
P.E	**L**	39	24	7	0	0
	S	`6	10	0	0	0

Table 4: Standard of Antifungal Activity

Standard of Antifungal Activity			
No	**Zone of inhibition (mm)**		
	Chaetomium	***A.niger***	***Neurospora***
1	25	28	30

Table 5: Antifungal activity of Gymnema sylvestre

Antifungal activity of *Gymnema sylvestre*							
NO	**SOLVENTS**	**Zone of inhibition (mm) *Gymnema sylvestre* leaf extract**			**Zone of inhibition (mm) *Gymnema sylvestre* stem extract**		
1	**DMSO**	22	20	22	-	28	24
2	**DMF**	-	-	-	-	-	-
3	**Acetone**	40	-	-	21	36	24
4	**Methano**	23	40	33	26	-	-
5	**D/W**	-	-	-	-	-	-
6	**P. ether**	-	-	-	-	-	-

Table 6: FTIR result of Gymnema sylvestre of branch

No	Obtain frequency (wavenumber)	Present group	No	Obtain frequency (wavenumber)	Present group
1	3344	Alcohol	**7**	1735	Ester
2	2917	Alkane	**8**	1718	Ketone
3	1802	Carbonyl	**9**	3344	Amine
4	2849	Amide	**10**	1718	Dimer
5	1718	Aldehyde	**11**	1734	Aldehyde & ketones
6	1825	Anhydrine	**12**	1731	Aldehyde & ketones

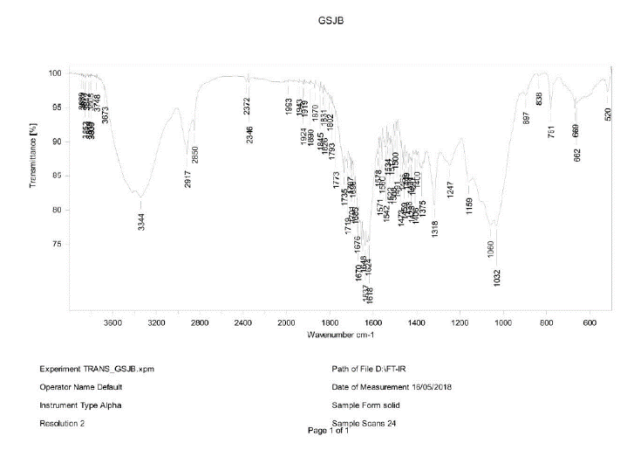

Figure 1: Result of FTIR extract of *Gymnema sylvestre* branch