Pharmacological studies of *Gymnemasylvestre leaves* extract

Abstract-

We have undertaken to evaluate the pharmacological studies of *Gymnemasylvestre*usingpharmacolicalmethods,disc diffusion, and anti-inflammatory methods. The Alkaloid, Carbohydrate, Protein, Resin, Saponin, and Glycosides were present whereas, steroid, flavanoid, and tannin were absent in phytochemical screening in *G. sylvestre* extract.The result showed induced paw edema using Carrageenan model..The dose dependent antibacterial was also observed. The present study is important because *Gymnemasylvestre*plant is important to cure various ailmentsin traditional medicine

Key Words: Antibacterial , Phytochemical, *Gymnemasylvestre, Antiinflammatory activity*

INTRODUCTION

The leaves of*Gymnema* reported a loss of sweet taste. The major chemical constituents of *Gymnema*aregymnemic acid and gurmarin. It was reported that Gymnemic acids have antidiabetic, anti sweetener, anti-inflammatory, antioxidant activities, and antidote properties.((Gloria *et al* 2003, Dateo et al, 1973, , Kanetkar*et al*, 2004, Persaud*etal*, 1999, Rachh*et al*, 2009, Puratchimani). *It helps* to promote weight loss possibly through its ability to reduce cravings for sweets and control blood sugar levels. The gurmarin peptide block the ability to taste sweet or bitter flavors and thus reduces sweet cravings (Preuss*etal*, 2004). The wound healing activity in albino mice. I*n vitro*, the inhibitory effects of DPPH radicals and LDL oxidation and muscle relaxant properties and antiallergic activity were reported (Kiranmai*et al*, 2011 , Luo *et al*, 1999 , Tandon*et al*, 2010). The radio protective and immune modulatory

Comment [Ma1]: Title should be more comprehensive. Add some more details. The title is not comprehensive and attractive. Please revise it. It must be improved stating the mechanisms/strategy adopted by the plant species.

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effect of Gymnemic acid was reported on Swiss albino mice induced by radiation The studies reported the anticancer activity of Gymenemasylvestre on MCF 7 (epithelial cells of human breast cancer and A 549 epithelial cells of human lung cancer under in vitro conditions by MTT assay method (Bhatia *et al,* 2008, Jitender*et al*, 2008 , 2009). Srikanth *etal*, 2010 and Sonam and Agrawal,2018).

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A clear working hypothesis should be introduced.

MATERIALS AND METHODS

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Extraction Process:-

Procedure:

The *GymnemasylvestreI*eaves were collected from the local garden of Chitrakoot Uttar Pradesh in the month of April 2020 and were identified by competent Botanist Dr .ManojTripathi of DRI, Chitrakoot. . The leaves were cleaned and dried for few days in shade. Then powder was made with the help of grinder. The extraction was done as per the method reported in earlier paper On the day of experimentation, the desired amount of powder was suspended in double distilled water for the final administration. Phytochemical screening was done as per method reported by Agrawal, 2021a,b and 2022)

1.2 Antibacterial Activity:-

The test organisms were obtained from the Department of Research, PBCRI Satna (M.P.).Antibacterial screening was done to find out the antibacterial properties of different concentration of 50% methanolic extract of *GymnemasylvestreI*eaves under study Kirby-Bauer Method (Disc diffusion method) was followed to test the antibacterial activity of different concentration of leaves extract.

Evaluation of Anti-inflammatory Activity*Carrageenin-induced rat paw oedema*

The mice were divided into five groups (n = 3). Acute inflammation was induced by the sub-plantar administration of 0.1 ml of 1% carrageenin in normal saline in the right hind paw of the mice. The paw volume was measured at 0 h and 4 h after carrageenin injection, using Plethysmometer.The different groups were made as described in the Table 4.The mice were divided into five groups (n = 3). After shaving the fur, the mice were anaesthetized under light ether and 10 mg of sterile

cotton pellets were inserted, one in each axilla of the mice. Extract (200, 300 and 500 mg/kg), Phenylbutazone (100 mg/kg) and to group control vehicle were administered orally for seven consecutive days from the day of cotton pellet implantation. The animals were anaesthetized on the eighth day and cotton was removed surgically. The pellets were dried at 60°C.The results were expressed as mean ± S.E.M. The significance statistical analysis was performed by t test and $P < 0.01$, implied significance organism was recorded.

RESULTS

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The present study revealed the presence of medicinally important bioactive compound. The phyotochemical screening of 50% methanolic extract of *Gymnemasylvestre* are depicted in table 1.The result showed the presence of Alkaloid, Carbohydrate, Protein, Resin, Saponin, Glycosides. Only Starch, steroid, flavanoid, tannin are absent in *G. sylvestre* extract.

Table 1: Qualitative Phyto-chemical Screening of (*Gymnemasylvestre*(Retz.) Schult. (Leaf)

S. No.	Name of Experiments	Observation	Result
1.	**Alkaloids**		
	a) Mayer' test	Yellow colour appear	Present
	b) Wagner's test	Brown colour appear	Present
	c) Dragendorff's test	Orange colour appear	Present
2.	**Carbohydrate**		
	a) Anthrone's test	Dark colour appear	Present
	b) Fehling's test	Green colour appear	Present
	c) Molisch's test	No red – violet ring disapper	Present
3.	**Proteins**		
	a) Bieuret's test	Green colour appear	Present
	b) Millon's test	White ppt are not appear	Present
5.	**Resins**	Turbidity are seen	Present
6.	**Saponins**	Honey comb – like structure are form	Present
7.	**Starch**	Red colour is formed	Absent

8.	**Flavonoid**		Absent
	a) Ferric chloride test	Reddis pink colour is appear	Absent
	b) Alkaline reagent test	On addition of dilute acid yellow col our disappear	Present
9.	**Steroid**		
	a) Salkowski's reaction	A red colour is disappear in the chlor oform layer	Absent
10.	**Glycoside**		
	a) Borntrager's Test	Colour is change	Present
11.	**Tannin**	Greenish colour appear	Absent
	a) Lead acetate Test	Reddish brown bulky ppt. are formed	Absent

2 Antibacterial assay

50% methanolic extract of *G. sylvestre* at the different concentration i.e. 25%, 50%, 75%, 100% exhibited antibacterial against *Bacillus subtilis* , *Staphylococcus aureus* but the lower activity was observed *E. coli* and *Pseudomonas aeroginosae* at 100%) the Minimum inhibitory concentration (MIC) of Gym. Sylvestre against gram positive bacteria i.e. *Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus* was 25% but against gram negative bacteria Zone of inhibition was observed only in 100% extract . Other concentration i.e. 25%, 50 %

Table2 Antibacterial activity of *Gym. Sylvestre* against bacterial strains

Name of microorganisms	**% Concentration of Extract [zone of inhibition(mm)]**			
	25	**50**	**75**	**100**
S.aureus	16	17	16	15
B.subtilis	10	12	13	15
E. Coli	13	14	13	15
Ps. aeroginosae	--	--	11	12

Figure 1 (a) Zone of inhibition of *Gymnema sylvestre* extract against different strains of bacteria.

Table 3. Antibacterial activity of standard antibiotic (gram positive) against different bact eria.

Name of microorganisms	**Name Standard antibiotics [zone of inhibition(mm)]**			
	TE	**OF**	**AZ**	**PC**
S.aureus	15	16	16	14
B.subtilis	14	16	18	14
S.epidermidis	14	18	17	17

TE- Tetracycline, OF- Ofloxacin, AZ- Azithromycin & PC- Piperacillin

Table 4 Antibacterial activity of standard antibiotic (gram negative) against different bacteria.

Name of microorganisms	**Name Standard antibiotics [zone of inhibition(mm)]**			
	FU	**GM**	**CX**	**NF**
E.coli	12	16	8.0	16
Sh.flexineri	18	18	12	21
P.aeruginosa	14	13	18	20

FU- Nitrofurantoin, GM- Gentamicin, CX- Cefotaxime & NF- Norfloxacin

S. aureus – *Staphylococcs aureus*,B. subtilis – *Bacillus subtilis*, S. epidermidis – *Staphylococcus epidermidis* ,E. coli – *Escherichia Coli* ,Sh. Flexineri – *Shigella flexineri*

P. Aeruginosa – *Pseudomonas aeroginosae*

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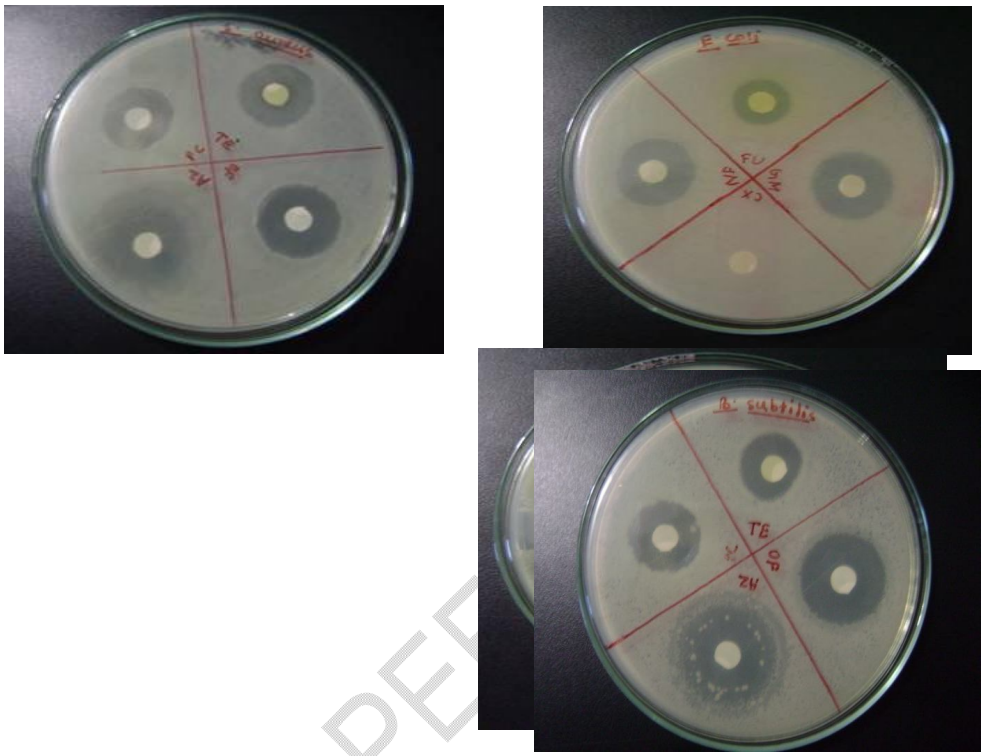


Figure 2 (b)Zone of inhibition of *standard antibacterial against* different strains of bacteria

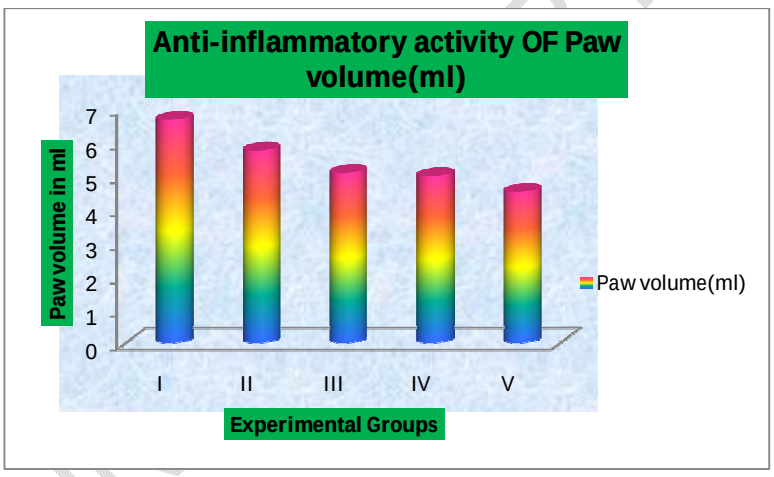
4 Anti-inflammatory activity-

The result of anti-inflammatory activity of methanol extract of *Gymnemasylvestre* on Carrageenan induced paw edema is shown in table 5. The methanolic extract of 400mg/kg decreased the paw edema volume by 28.69% within 4 hour after administration, while standard drug decreased the paw edema volume by 46.05% when compared with the paw edema volume

of control. The gymnemasylvestre extract at the dose of 200, 300, 400 mg/kg produced significant reduction, when compared to the control group.

Table 5. valuation of anti-inflammatory activity of *Gymnemasylvestre*leaves extract

S.No.	Group	Paw volume(ml)	%inhibition
1.	Control	6.66±0.08	
2.	Gymnema sylvestre extract(200mg/kg)	5.74±0.11	16.95
3.	Gymnema sylvestre extract(300mg/kg)	5.07±0.05	22.16
4.	Gymnema sylvestre extract(400mg/kg)	4.98±0.08	28.69
5.	Diclofenac sodium(100mg/kg)	4.51±0.12	46.05



Graph 1 Effect of *G.sylvestre* extract on Antiinflammatory activity of Paw volume(ml)

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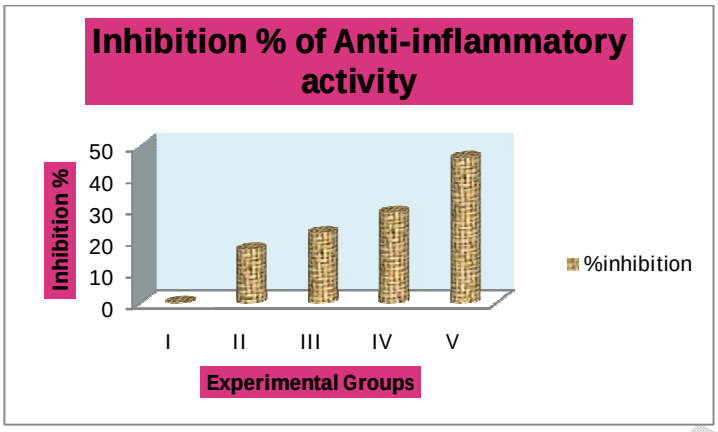

Graph2 Effect of *G.sylvestre* extract on inhibition % of Anti - inflammatory activity

DISCUSSION AND CONCLUSIONS

. Present study showed that the extract of Gymnemasylvestre extract caused antimicrobial activity against gram positive and gram negative bacteria .It also showed anti inflammatory activity in mive. It is also reported the significant anticancer activity and antimicrobial . The study is important for scientific community because this plant is used in traditional medicine to treat various diseases. .

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Comment [Ma12]: Too short.to evaluate. Discussion is weak. The discussion needs enhancement with real explanations not only agreements and disagreements. Authors should improve it by the demonstration of biochemical/physiological causes of obtained results. Instead of just justifying results, results should be interpreted, explained to appropriately elaborate inferences. Discussion seems to be poor, didn't give good explanations of the results obtained. I think that it must be really improved. Where possible please discuss potential mechanisms behind your observations. You should also expand the links with prior publications in the area, but try to be careful to not over-reach. For the latter, you should highlight potential areas of future study.

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English should improve by a native person.
The paper suffers from a poor English structure throughout and cannot be published or reviewed properly in the current format. The manuscript requires a thorough proofread by a native person whose first language is English. The instances of the problem are numerous and this reviewer cannot individually mention them. It is the responsibility of the author (s) to present their work in an acceptable format. Unless the paper is in a reasonable format, it should not have been submitted.
The list of references should be carefully checked and corrected.

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