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Study of different pharmacological activities of bark extracts of *Grewia multiflora* Juss.

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ABSTRACT

The decoction of bark of *Grewia multiflora* Juss. (Malvaceae) claimed to be used for healing of wounds, treating microbial infections, inflammation, pain and worm infestation. In this study, bark powder was extracted following soxhlation technique to get acetone, methanol and purified water extracts and also methanol(70) extract by extracting residue remained after chloroform extract by a mixture of methanol(70%) and purified water(30%). The yield of B-Me(70) was higher than other extracts. B-Me(70) extract was found to contain maximum number of phytoconstituents due its maximum solvent properties. Methanol and methanol(70) extracts were selected for investigation of antimicrobial, wound healing, anti-inflammatory, analgesic and anthelmintic activities. Ointment of extracts in Hydrophilic ointment USP base was used to study antibacterial activity by agar cup method comparing with the standard antibacterial (Soframycin cream). B-Me(70) at 15% concentration showed higher zone of inhibition comparable to standard. This ointment was taken to study wound healing potential by excision wound model. 18 days were taken for complete closure of wound by 15 % w/w of B-Me(70) extract in comparison to standard within 15 days. B-Me(70) extract exhibited anthelmintic activity. Anti-inflammatory activity was studied by Carrageenan, Arachidonic acid and Histamine induced rat paw edema method. B-Me(70) extract (300 mg/kg) showed 56. 96%, 55.17% and 58.28% inhibition of edema respectively. B-Me(70) (300 mg/kg) showed analgesic effect ($p < 0.01$) when tested by hot plate method and also by acetic acid induced writhing method($p < 0.05$).

KEY TERMS: *Grewia multiflora* Juss., bark extract, Methanol(70), Hydrophilic ointment USP, antimicrobial activity, anti-inflammatory, analgesic.

INTRODUCTION

Vaidyas and local tribes are using the decoction of bark of *Grewia multiflora* Juss for healing of wound, treating microbial infections, inflammation, pain and worm infestation. *Grewia multiflora* Juss, a plant grown in the biosphere of Similipal forest territory was selected for study. The plant is called Bhansuli in Hindi and Hadajodi in Odiya.[1] *Grewia multiflora* Juss. is found spread throughout our country and other parts of world.[2] Bark is used in inflammation, pain, fever, cure of wounds in Bangladesh, used for rheumatism and also in various psychological disorders.[3,4] Bark contains phenols, saponins, flavonoids. Alkaloids, terpenoids, steroids, anthocyanins, fatty acids, keto alcohols etc.[5,6] Bark extracts of *Grewia multiflora* Juss. were studied pharmacologically. The plan was to study the analgesic, anti-inflammatory, anthelmintic, antibacterial and wound healing activities of bark extracts.

MATERIAL AND METHOD:

Pharmaceutical high grade chemicals were purchased /procured.

Plant material procurement:

Stem bark of *Grewia multiflora* Juss was cut and removed from the trunk of the plant freshly(5kg) from the Meru Matha area near Subarnarekha river in the biodiversity territory of Similipal forest, dist- Mayurbhanj, Odisha. in August 2018 (Fig.1). We took the help of Botanical survey of India, Howrah, WB for authentication.. A sample specimen was deposited in that herbarium.

Extraction:[7]

Method: Freshly collected barks were cleaned from wood particles manually and cut in to small pieces. Bark pieces were dried away from sun light in a dry place. Dried pieces of bark were grinded thoroughly to get moderately coarse powder. The powder was subjected to Soxhlation first by petroleum ether (60-80 ^{o}C) to make free from fatty substance and chlorophyll etc. The residue was then successively extracted by chloroform (Cm). Residue remained after extraction with chloroform was divided into two portions. One portion was subjected to successive extraction by acetone (Ac), methanol (Me) and purified water (Aq). The other portion was extracted with methanol (70) [a mixture of two solvents containing

methanol (70%) and purified water (30%)].[8] The physical parameters of extracts are given in Table 1.

Test for presence of phytoconstituents:[9]

Different established methods(as mentioned in the result table) were adopted for analysis of secondary metabolites in the extracts. The result of phyto-analysis is shown in Table 2.

Flavonoid Content estimation (TFC):[10]

Method: Modified $ALCl_3$ spectroscopic method was followed. TFC of extracts was counted in mg/100gm of quercetin. The observations are noted in Table 3 and shown in Fig.2.

Phenolic Content estimation(TPC):[11]

Method: TPC was accounted as mg/100gm of gallic acid when estimated by a method using Folin-Ciocalteu Reagent. The observations are noted in Table 3 and shown in Fig. 2.

Acute Toxicity Study:[12]

Healthy Wistar albino rats of 200 gm weight (average) were maintained in standard approved conditions of light and darkness, temperature and humidity. Animals were acquainted to climatic and room conditions 4 days before test. CPCSEA necessities were fulfilled. The author had received the IAEC approval from SIPS, Jharpokharia, Mayurbhanj, Odisha, India (Regd.No.:-787/PO/ac/03/CPCSEA) for all experimental protocols, Vide approval No: A09/18/IAEC/SIPS. Acute toxicity study was conducted.

Method: OECD guidelines was taken for study. Twenty five (25) gram of Methanol(70) bark extract was accurately weighed and 0.5% Na CMC solution was mixed, stirred to get 100ml of suspension. Suspensions of 0.4mL,08mL,1.6mL,2.4mL, 3.2mL, 4 mL 8 mL and 12mL respectively [at doses of 100mg, 200mg, 400mg, 600mg, 800mg, 1000mg, 2000mg/kg and 3000mg/kg] were fed to separate groups of rats(n=6). 10 ml/kg b.w. (0.5 % W/V Na CMC) in distilled water was fed orally as control. The animals were observed for toxic reactions from 3 hrs till daily once for 7 days and extended to 20 days. The rats were examined for mortality, toxicity and behavior.

Selection of extracts for further study: Considering high yield, maximum content of phytochemicals, higher content of total flavonoids and phenolics B-Me(70) and B-Me of bark were taken for pharmacological investigations. 150mg/kg and 300mg/kg body weight per oral

as the lower($1/20^{th}$) and higher($1/10^{th}$) therapeutic dose respectively were selected for further pharmacological studies.

Preparations for study of pharmacological potential:

Selection & grouping of animals: For Analgesic and anti-Inflammatory activity study, healthy rodents [Swiss Albino mice (25-35 gm) and Wistar albino rats (180-225 gm)] were taken respectively in six numbers per group. Rodents were acquired from the Institution. Adult female and male mice and rats were selected and maintained in standard approved conditions for study. Mice/rats were grouped as:

Gr.-I : Control (1 mL of 0.5 % Na CMC solution)
Gr.-II : Standard [Aceclofenac (10 mg/kg)]
Gr.-III : B-Me(150 mg/kg)
Gr.-IV : B-Me(300 mg/kg)
Gr.-V : B-Me(70)(150 mg/kg)
Gr.-VI : B-Me(70)(300 mg/kg)

Preparation of 0.5% Na CMC solution: 0.5 gm of Na CMC was dissolved in purified water to get 100mL of solution. 1mL was fed to rodents as control orally during study.

Preparation of extract suspension for mice: As the dose of extracts were 300mg/kg or 150mg/kg, the amount per mice (30 gm body weight approximately) was calculated to be 9 mg or 4.5 mg respectively. 900 mg or 450 mg of extract was taken, stirred with the control liquid to get 100ml of each dispersion. Thus, the extract suspension contained 9 mg/mL or 4.5 mg/mL.

Preparation of extract suspension For rat: As the dose of extracts were 300mg/kg or 150mg/kg, the amount per rat (200 gm body weight approximately) was calculated to be 60 mg or 30 mg respectively. 6 gm or 3 gm of extract was taken, stirred with the control liquid to get 100ml of each dispersion. Thus, the extract suspension contained 60 mg/mL or 30 mg/mL.

Preparation of drug suspension for mice: The dose of aceclofenac to be administered is 10 mg/kg (0.3 mg/mL for 30 gm weight mice). 30 mg of drug was added to 0.5% Na CMC solution(control liquid) to make 100mL of suspension. Each mL contained 0.3 mg of aceclofenac.

Preparation of drug suspension for rat: The dose of aceclofenac to be administered is 10 mg/kg (2 mg/mL for 200 gm weight rat). 200 mg of drug was added to 0.5% Na CMC solution (control liquid) to make 100mL of suspension. Each mL contained 2 mg of aceclofenac.

Analgesic Activity investigation:

Hot plate method:[13] Overnight fasted, healthy adult mice were taken (6xn=6). Gr-1 fed 1 mL of control vehicle orally. Gr-2 served as standard and Gr-III to VI served as test group fed with 1mL of extract preparations as mentioned above orally. The hot plate was heated at 55±1 ^{o}C, paw of mice were put on the plate. The time taken to withdraw the paws was noted before and latter 30 minutes interval up to 2.5 hours. The results were given in Table-4 and Fig.3.

Acetic acid induced writhing test:[14] Mice were grouped and fed with vehicle/ drug/extract preparations each 1 mL orally as mentioned under hot plate method. After 30 minutes, aqueous acetic acid (0.6%) as writhing agent was injected i.p. [dose-10 mL/kg (b.w.)]. Writhing produced in animals were counted for next 30 minutes and computed by comparison with control. The result was computed as % protection and given in Table 5 and Fig.4.

$$\% \text{ protection} = \frac{NWC - NWT}{NWC} \times 100 \qquad \text{……. Eq-1}$$

Where, NWC is number of writhes in control and NWT is number of writhes in test.

Anti-Inflammatory Activity investigation:

Different rat paw edema models were selected using inducing agents like Carrageenan, Arachidonic acid and Histamine.

Induction by Carrageenan:[15] Rodents were grouped (6x6) and properly maintained. The rodents were fed with vehicle/ drug/extract preparations each 1 mL orally as mentioned above. Thirty minutes after administration of the respective extracts and aceclofenac, 0.1 mL carrageenan (1% solution in 0.9% saline solution) was injected into the planter region of left hind paw of rats. Paw volume was measured by using digital plethysmometer. The anti-inflammatory activity was determined in terms of percent edema inhibition which was calculated and recorded in Table-6 and shown in terms of % inhibition in Fig.5.

$$\% \text{ Inhibition of paw edema} = \frac{PEC - PET}{PEC} \times 100 \quad \text{.....Eq.2.}$$

Where, PEC =Paw edema (control) and PET =Paw edema(treated group)

Induction by Arachidonic acid:[16] Rodents were grouped (6x6) and properly maintained. The rodents were fed with vehicle/ drug/extract preparations each 1 mL orally as mentioned above. 0.5% (w/v) arachidonic acid solution was made in 0.2 M carbonate buffer having pH 9.2. Thirty minutes after administration of the respective extracts and aceclofenac, 0.1mL of above solution was injected and paw volume was measured as above. The observation as % inhibition was documented, calculated and recorded in Table-6 and shown in terms of % inhibition in Fig.5.

Induction by Histamine:[16] Rodents were grouped (6x6) and properly maintained. The rodents were fed with vehicle/ drug/extract preparations each 1 mL orally as mentioned above. Thirty minutes after administration of the respective extracts and aceclofenac, histamine solution (0.1%w/v in normal saline) was injected into the paw of each rat at a dose of 0.1mL to induce edema and paw volume was measured as above The observation as % inhibition was documented, calculated and recorded in Table-6 and shown in terms of % inhibition in Fig.5.

Anthelmintic Activity investigation:[17]

Arrangement for experiment: Anthelmintic activity was tried using *Pheretima posthuma* L. Vaill (Annelida) obtained from Horticulture Department, Baripada, Dist Mayrurbhanj, Odisha of average size (8 cm). The worms were freed from fecal matter by washing with normal saline. Piperazine citrate was used as standard drug as 10 mg/mL in control solution. Na CMC(0.5%) solution in normal saline was served as control. B-Me and B-Me(70) extract of *Grewia multiflora* Juss were dispersed in control solution to get 50 mL each having concentrations of 50 and 100 mg/mL.

Method:[18] Six petri dish of nine cm diameter were taken. 50ml of each concentration of extract solution, standard solution and control were poured in the petri dish respectively. Six worms (8 cm approximately) were placed in each petri dish. Observations were recorded for average paralysis time and death time of worms in Table-7.

Antibacterial Activity investigation:[19]

Test bacteria: *Staphylococcus aureus* (SA), *Bacillus licheniformis*(BL) as Gr+ve and *Escherichia coli*(EC), *Salmonella typhimurium*(ST), *Shigella sonnei*(SS),*Vibrio cholerae*(VC), *Vibrio alginolyticus*(VA), *Shigella boydii*(SB) as Gr-ve bacteria were obtained from Jadavpur University, Department of Pharmaceutical Technology, Calcutta, WB and preserved as slant cultures at a temperature of 4 ^{o}C in our laboratory with proper maintenance.

Preparation of ointment of extracts: Selected bark extracts [B-Me and B-Me(70)] of *Grewia multiflora* Juss were triturated with 2.5% DMSO solution. The dispersion was mixed with the Hydrophilic Ointment base USP(prepared as per official method) and triturated in a mortar to get the herbal ointments (10% and 15%w/w).

Method: Nutrient Agar (NA) media was prepared, sterilized and poured into sterile petri dishes to obtain NA plates having approximately 4 mm uniform thickness. Overnight Nutrient Broth (NB) cultures of the respective test organisms were firmly seeded over the respective agar plate surfaces using a sterile cotton swab so as to make a lawn. Six holes of 6 mm diameter were punched on these inoculated plates using sterile gel puncher. Prepared ointments of bark extracts with standard (Soframycin cream) and control(the base with 2.5% DMSO solution) were filled in the holes in the plates. The petri dishes were incubated(at 37 °C for 24 hrs). All the operations were carried out under aseptic environment. The plates were studied for clear ZOI around each hole and zone diameters were measured. Average zones of inhibition(n=3) were recorded in Table-8 and shown in Fig.6.

Wound Healing Activity investigation:[20]

Considering the higher antibacterial activity, ointment of B-Me(70)) containing 15% extract was investigated towards its wound healing potential in contrast to the standard Soframycin cream 1%.

Grouping of animals: Wound healing potential was measured by Excision wound model using rats (180 – 225 gm.). Rodents were kept in animal room well maintained for seven days with proper diet and water. The rodents were grouped (4x n=6) as

Gr. I : Untreated

Gr. II : Control (ointment base with 2.5% DMSO).

Gr. III : Standard (Soframycin cream 1%)

Gr. IV : B-Me(70) ointment containing 15% of extract

Method: Back skin of the rodents was shaved, cleaned, disinfected with 70 % alcohol. The surface was encirculated by methylene blue. Wounds (approximately area 280-325 mm^2 and 2 mm depth) on back of anaesthetized rodents were made by removing the skin using a surgical blade. The wound area was left undressed to open environment. Gr. I rodents were left untreated. Wound of Gr. II were treated with application of ointment base with 2.5% DMSO (control). Soframycin cream was applied to wound of Gr. III rodents once daily. B-Me(70) ointment containing 15% of extract was applied to wounds of Gr. IV rodents once daily. Progressive contraction of wound was measured by taking the area on a graph paper and evaluated at an interval of 3 days till complete closure. % calculation of wound contraction was made and recorded in Table- 9 and shown in Fig. 7. Effect of the ointment of extracts on % contraction of excision wounds is tabulated in Table- 10 and shown in Fig. 8.

RESULT & DISCUSSION:

The amount of B-Me(70) extract was found to be higher (14.21%)) than B-Me (8.85%).Other extracts were having low yield. Most of the phytoconstituents were extracted in the mixed solvent [methanol(70)]. Both B-Me(70) and B-Me extract showed positive test for presence of alkaloids, glycosides, saponins, flavonoids, steroids, terpenoids, phenolics, carotenoids and coumarins.

TPC and TFC: Total Flavonoid & Phenolic content of B-Me(70) was higher than B-Me. Others contained less amount of flavonoids & phenolics.

Toxicity findings: As because there was no mortality up to the dose of 3000 mg/kg B.W., the lethal dose of the extracts was quite high. The plant is safe for use orally. 150mg/kg and 300mg/kg B.W. per oral as the lower($1/20^{th}$) and higher($1/10^{th}$) therapeutic dose respectively were selected for further pharmacological studies.

Selection of extracts for further study: In case of bark extracts of *Grewia multiflora* Juss, it was found that the yield % was higher in B-Me(70) than B-Me. Other extracts such as acetone and aqueous extract had very negligible yield. Similarly maximum phytochemicals were extracted in B-Me(70) followed by B-Me. Other two extracts contained less number of phytochemicals. Both B-Me(70) and B-Me extracts were rich in flavonoids and phenolic compounds. It was observed that the B-Me(70) extract was non toxic and safe to use in the quantity up to 3000mg/kg b.w. Thus it was decided to proceed further with B-Me(70) and B-Me for pharmacological investigation.

Analgesic Activity assessment:

Both hot plate and acetic acid induced writhing method were executed to evaluate the analgesic effect of selected bark extracts of *Grewia multiflora* Juss. B-Me(70)[300 mg/kg] showed maximum effect($p < 0.01$) whereas B-Me[300 mg/kg] showed lesser effect ($p < 0.05$).

Anti-Inflammatory Activity assessment:

Carrageenan, arachidonic acid and histamine induced rat paw edema methods were adopted for anti-inflammatory activity assessment and it was found that in all the model, B-Me(70)[300mg/kg] showed significant % of inhibition than B-Me[300mg/kg]. Extracts(150mg/kg) showed non-significant results. B-Me(70)[300mg/kg] showed 56.96%, 55.17% and 58.28% inhibition whereas B-Me[300mg/kg] showed 46.84%,42.86% and 51.37% inhibition at 4^{th} hour respectively.

Anthelmintic Activity assessment:

Time of paralysis(15.71minutes) and death(27.31 minute) was recorded with B-Me(70) extract of *Grewia multiflora* Juss @100 mg/mL dose. The corresponding B-Me extract was taken the time for paralysis(24.17minutes) and death (31.26 minute) with above dose. Anthelmintic activity of B-Me(70) [100mg/mL] was higher than the standard piperazine citrate (10mg/mL).

Antibacterial Activity assessment:

Zone of inhibition obtained by ointment of B-Me(70) with 15% extract was higher than that of ointment of B-Me(70) with 10% extract. Ointment of B-Me(70) extract(15%) showed significant($p<0.01$) result producing the ZOI from 25.17 to 26.69 mm against the tested bacteria whereas Ointment of B-Me(70) extract(10%) showed the ZOI from 19.08 to 21.75 mm which was non-significant. However, Ointment of B-Me extract (15%) showed significant($p<0.05$) result producing the ZOI from 21.54 to 23.12 mm against the tested bacteria. Ointments containing 15 % of B-Me(70) extract which showed better ZOI in comparison to the standard soframycin cream (ZOI being 27.07 to 29.03 mm against the tested bacteria) was selected for wound healing activity study.

Wound Healing Activity assessment:

A remarkable wound repairing within 18 days was observed with B-Me(70)[15%] ointment whereas the standard antibacterial(soframycin cream) showed complete wound healing within 15th day.

Fig. 1. Bark of *Grewia multiflora* Juss.

Table-1. Physical parameters of bark extracts of *Grewia multiflora* Juss:

Sl. No.	Type of extract	Code	Colour	Consistency	% yield
1	Chloroform	B-Cm	Brownish black	Pilular	0.96 %
2	Acetone	B-Ac	Brownish	Pilular	1.83 %
3	Methanol	B-Me	Brownish	Soft	8.85 %
4	Aqueous	B-Aq	Brownish	Soft	6.74%
5	Methanol(70)	B-Me(70)	Brownish black	Soft	14.21%

Table-2: Preliminary phytochemical screening of bark extracts:

Phyto chemicals	Test	B-Cm	B-Ac	B-Me	B-Aq	B-Me(70)
Alkaloids	Dragendroff	-	+	-	-	+
Glycosides	Legal	-	+	+	+	+
Saponin glycosides	Haemolysis	-	-	+	+	+
Flavonoid glycoside	Shinoda	-	-	+	+	+
Saponins	Foam	-	-	+	+	+
Flavonoids	Alkaline	-	+	+	-	+

Steroids	Liebermann -Burchard	+	+	+	-	+
Terpenoids		+	-	+	-	+
Reducing sugars	Benedict's	-	-	+	+	+
Carbohydrates	Molisch	-	-	-	+	+
Tannins	Gelatin	-	-	+	+	+
Amino acids & Proteins	Ninhydrin	-	-	+	+	+
Phenolic compounds	Iodine	-	+	+	-	+
Carotenoids	Carr-price	+	+	+	-	+
Coumarins	NaOH	-	-	+	-	+

Table-3: TFC and TPC of bark extracts:

Bark Extract	TFC (mg/100gm) as QE	TPC (mg/100gm) as GAE
B-Cm	0.32±0.05	0.35±0.01
B-Ac	45.15±3.28	25.14±2.19
B-Me	101.36±3.92	101.42±3.47
B-Aq	01.12±0.74	02.04±0.18
B-Me(70)	103.47±3.26	114.23±3.85

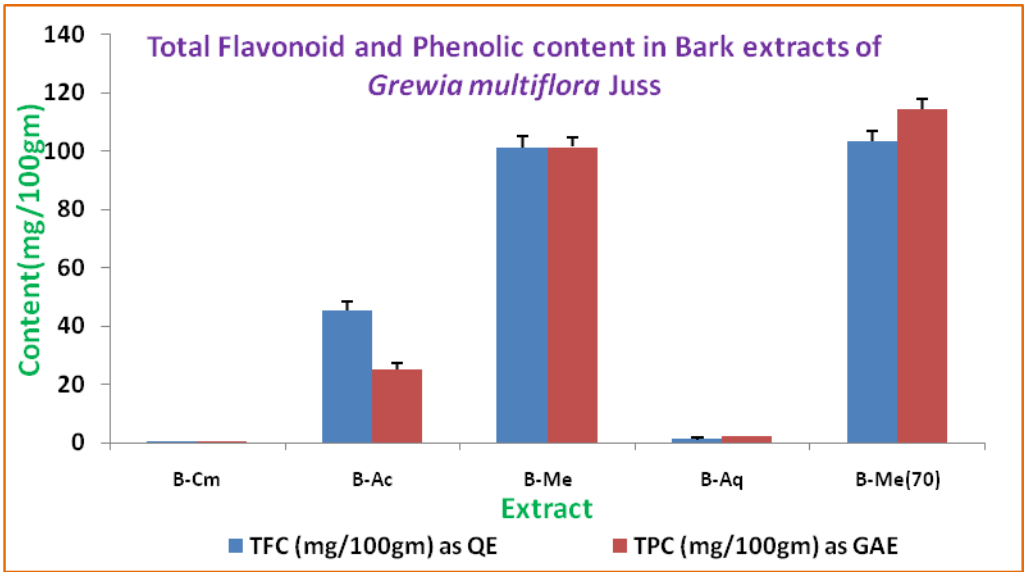

Fig. 2. Histogram showing TFC & TPC of Bark extracts of *Grewia multiflora* Juss.

Table -4: Analgesic activity shown by bark extracts by hot plate method:

Treatment	Gr n=6	Dose/ Kg b. w.	Basal reaction time	Reaction time (in sec) [Mean± SEM] after (minutes)			
				30	60	120	180
Control (Na CMC)	1	10ml	4.5± 0.115	5.0± 0.208	4.6± 0.148	4.8± 0.143	5.1± 0.149
Aceclofenac	2	10mg	4.7± 0.128	9.7± 0.057*	9.8± 0.113^	9.9± 0.101^	9.9± 0.114^
B-Me	3	150mg	5.0± 0.103	5.8± 0.115	6.9± 0.102	7.6± 0.057*	7.9± 0.100*
B-Me	4	300mg	4.9± 0.105	7.7± 0.154*	9.1± 0.115*	9.9± 0.308*	10.0± 0.152^
B-Me(70)	5	150mg	4.9± 0.103	6.1± 0.057	6.3± 0.052	6.5± 0.113	7.7± 0.120*
B-Me(70)	6	300mg	4.8± 0.155	8.2± 0.208*	8.7± 0.173^	9.1± 0.185^	9.2± 0.057^

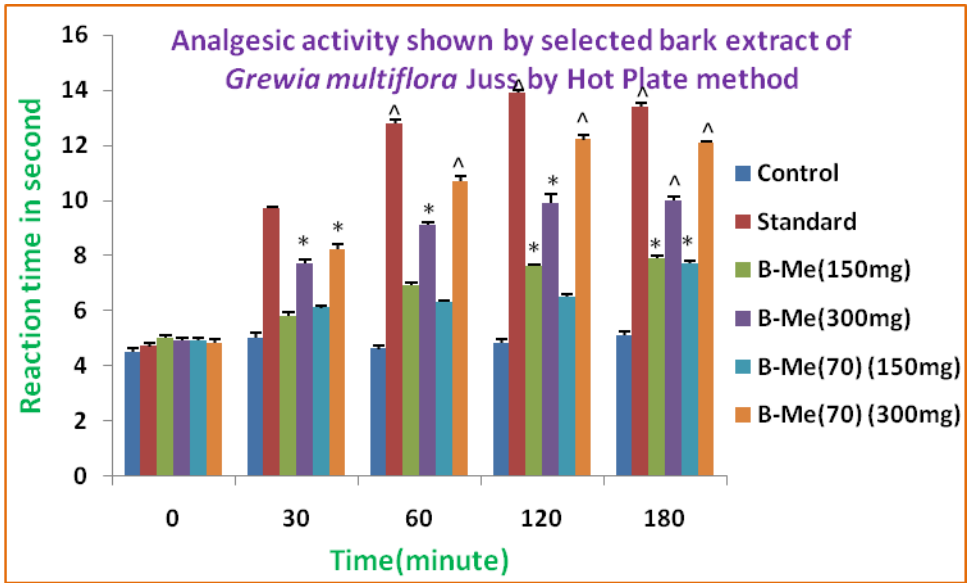

Fig.3. Analgesic activity shown by bark extracts by hot plate method.

Table-5: Analgesic activity shown by bark extracts by acetic acid induced writhing method:

Treatment	Gr n=6	Dose/ Kg b. w.	No. of Writhing in 30 min[Mean± SEM]	%Inhibition
Control	1	10mL	49.36± 0.59	-
Aceclofenac	2	10mg	6.05± 1.26^	87.74 %
B-Me	3	150mg	28.17± 2.16	42.93 %
B-Me	4	300mg	11.68± 0.89*	76.34 %
B-Me(70)	5	150mg	25.41± 1.20	48.52 %
B-Me(70)	6	300mg	8.09± 0.76^	83.61 %

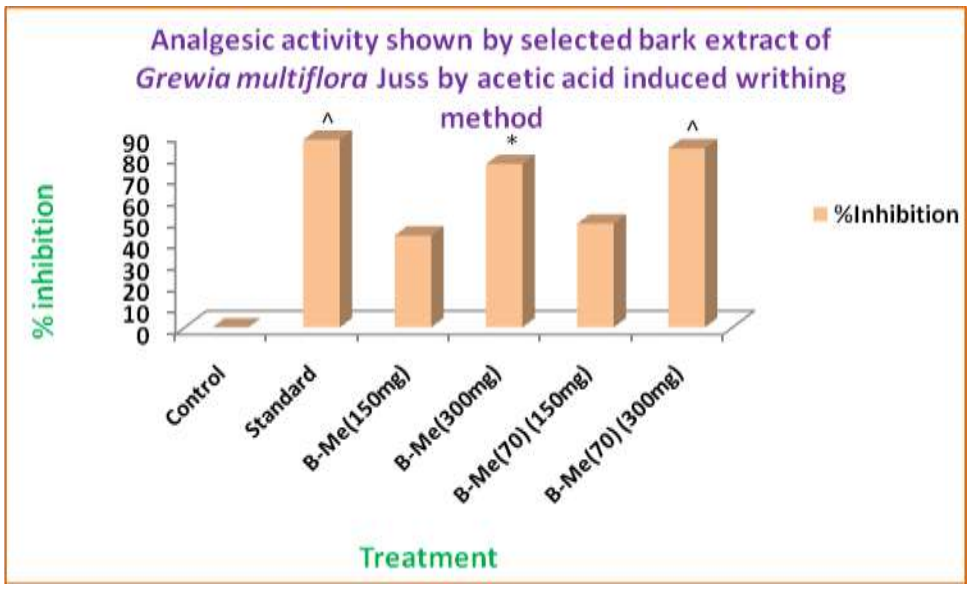

Fig.4. Analgesic activity shown by bark extracts by acetic acid induced writhing method.

Table-6: Anti-inflammatory effect of bark extracts on %inhibition at 4th hr of paw edema in rats induced by carrageenan, arachidonic acid and histamine:

Treatment	Gr [n=6]	Dose/ kg b.w.	% Inhibition of paw edema at 4th hr [Mean± SEM]		
			Carrageenan induced	Arachidonic acid induced	Histamine induced
Control	1	10mL	-	-	-
Standard (aceclofenac)	2	10mg	60.76 ± 0.87^	59.18 ± 0.34^	61.08 ± 0.85^
B-Me	3	150mg	11.39 ± 1.13	10.26 ± 0.38	20.60± 0.39
B-Me	4	300mg	46.84 ± 0.87*	42.86 ± 0.97*	51.37 ± 0.87*
B-Me(70)	5	150mg	16.46 ± 1.03	12.31 ± 0.13	26.33± 0.58
B-Me(70)	6	300mg	56.96 ± 0.42^	55.17 ± 0.63^	58.28 ± 0.79^

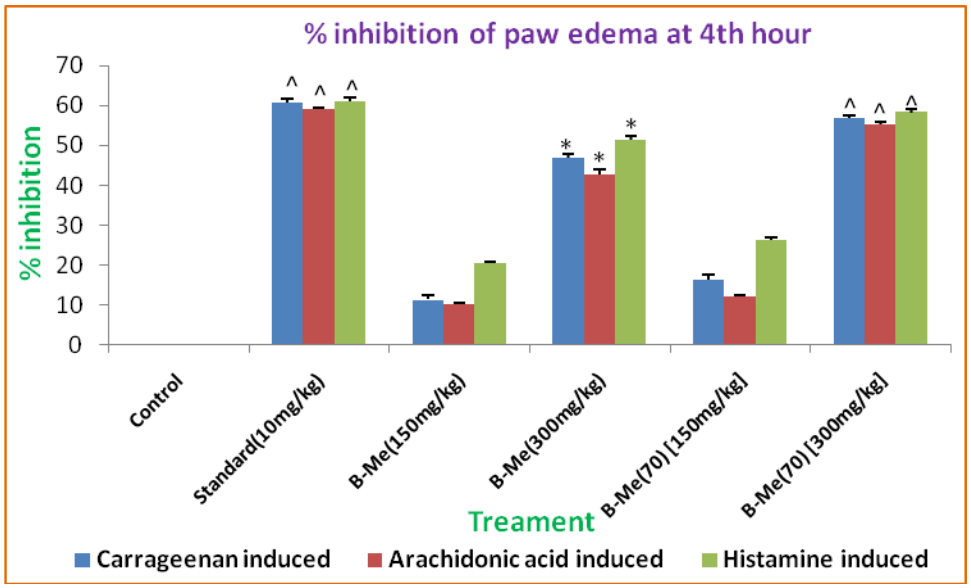

Fig .5. Anti-inflammatory effect of bark extracts on %inhibition at 4th hr of paw edema in rats induced by carrageenan, arachidonic acid and histamine:

Table-7: Anthelmintic activity shown by bark extracts of *Grewia multiflora* Juss:

Test sample	Dose in mg/mL	Time in minutes	
		Paralysis	death
Control	Na CMC solution	-	-
Piperazine citrate	10mg	20.28± 0.56	40.70± 0.53
B-Me	50 mg	33.43± 0.15	43.13± 0.26
B-Me	100 mg	24.17± 0.52	31.26± 0.15
B-Me(70)	50 mg	28.46± 0.32	43.10± 0.17
B-Me(70)	100 mg	15.71± 0.15	27.31± 0.37

Result were expressed in mean ±SD, n =6

Table-8: ZOI (in mm) shown by ointments of bark extracts of *Grewia multiflora* Juss against bacterial strains:

Sr. No.	Bacterial Strains	Zone of inhibition in mm[n=3] (Values are expressed in Mean± SEM)					
		Control	STD[@]	Ointment			
				B-Me (10%)	B-Me (15%)	B-Me(70) [10%]	B-Me(70) [15%]
1	SA	6.00± 0.00	29.03 ±0.36^	18.06± 0.24	23.12± 0.42*	19.08± 0.96	26.12± 0.74^
2	BL	6.00± 0.00	28.13 ±0.14^	18.63± 0.09	22.35± 0.31*	20.16± 0.42	25.53± 0.21^
3	EC	6.00± 0.00	28.31 ±0.27^	17.41± 0.23	22.45± 0.24*	21.75± 0.13	25.64± 0.30^
4	ST	6.00± 0.00	27.41 ±0.56^	17.75± 0.14	22.78± 0.13*	21.64± 0.11	26.18± 0.43^
5	SS	6.00± 0.00	28.04 ±0.35^	18.09± 0.17	21.54± 0.31*	20.53± 0.06	26.69± 0.07^
6	VC	6.00± 0.00	27.07 ±0.26^	18.35± 0.61	22.76± 0.42*	19.42± 0.31	25.07± 0.52^
7	VA	6.00± 0.00	28.21 ±0.13^	18.29± 0.14	22.18± 0.62*	19.28± 0.71	25.17± 0.32^
8	SB	6.00± 0.00	28.09 ±0.43^	17.42± 0.36	23.11± 0.38*	19.63± 0.34	26.32± 0.09^

[@] STD- Soframycin cream 1% (Aventis) was used as standard.

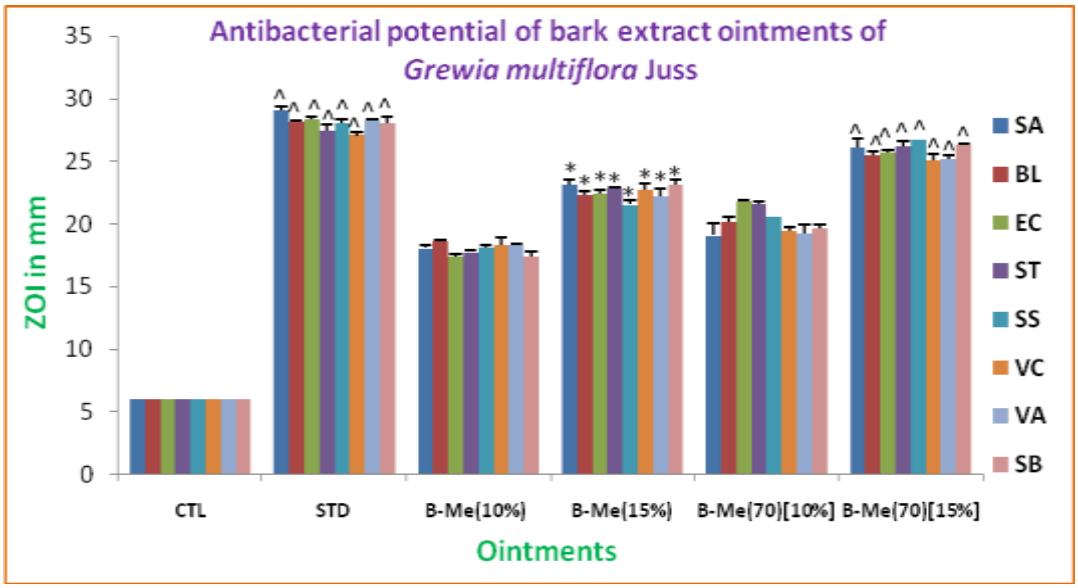

Fig.6. Histogram depicting the comparative ZOI shown by ointment of bark extract against bacterial strains

Table-9: Effect of ointments of B-Me(70) extract(15%) on excision wound area:

Post wound healing (Days)	Wounding area in(mm^2)[n=6] (Values are expressed in Mean± SEM)			
	Gr. I	Gr. II	Gr. III	Gr. IV
	Untreated	Control	Standard	B-Me(70)
0	306.03 ± 4.3	308.14 ± 3.8	303.42 ± 3.4	304.07 ± 1.9
3	280.25 ± 3.8	268.74 ± 1.9	248.69 ± 2.7	254.53 ± 2.8
6	242.23 ± 3.5	222.72 ± 3.8	182.13 ± 4.9*	192.61 ± 3.6*
9	203.07 ± 6.2	181.03 ± 4.2	108.14 ± 3.7^	124.11 ± 3.4*
12	151.21 ± 4.3	121.36 ± 3.7	32.47 ± 3.9^	46.07 ± 3.8^
15	111.23 ± 4.2	87.36 ± 4.1	0	23.12 ± 2.1^
18	73.14 ± 2.3	36.25 ± 3.6	0	0

Control- Hydrophilic ointment; Standard- Soframycin cream 1% (Aventis)

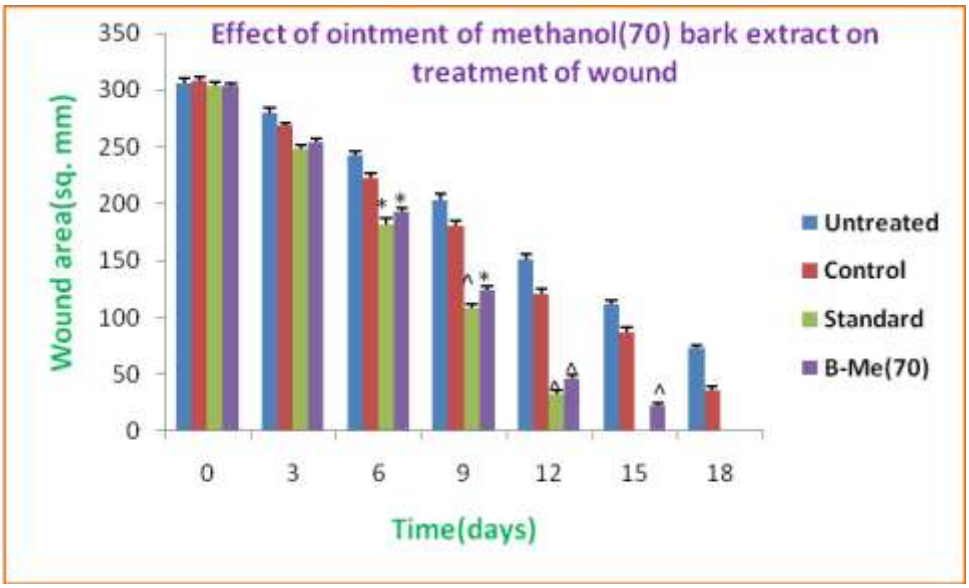

Fig.7. Effect of ointments of B-Me(70) extract(15%) on contraction of excision wound area.

Table- 10: Effect of ointments of B-Me(70) extract(15%) on % contraction of excision wounds:

Post wound healing (Days)	% Wound Contraction of Excision Wounds ± SEM(n=6)			
	Gr. I	Gr. II	Gr. III	Gr. V
	Untreated	Control	Standard	B-Me(70)
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
3	8.42±2.4	12.79±0.6	18.03±1.7	16.29±2.3
6	20.84±0.9	27.72±1.1	39.97±1.9	36.66±0.3
9	33.64±2.7	41.25±0.7	64.36±0.6*	59.18±1.1*
12	50.59±1.8	60.62±1.3	88.29±0.8^	84.85±0.8^
15	63.65±1.2	71.65±1.3	100±0.0^	92.40±1.1^
18	76.10±1.4	88.24±0.7	100±0.0^	100±0.0^

Control- Hydrophilic ointment; Standard- Soframycin cream 1% (Aventis)

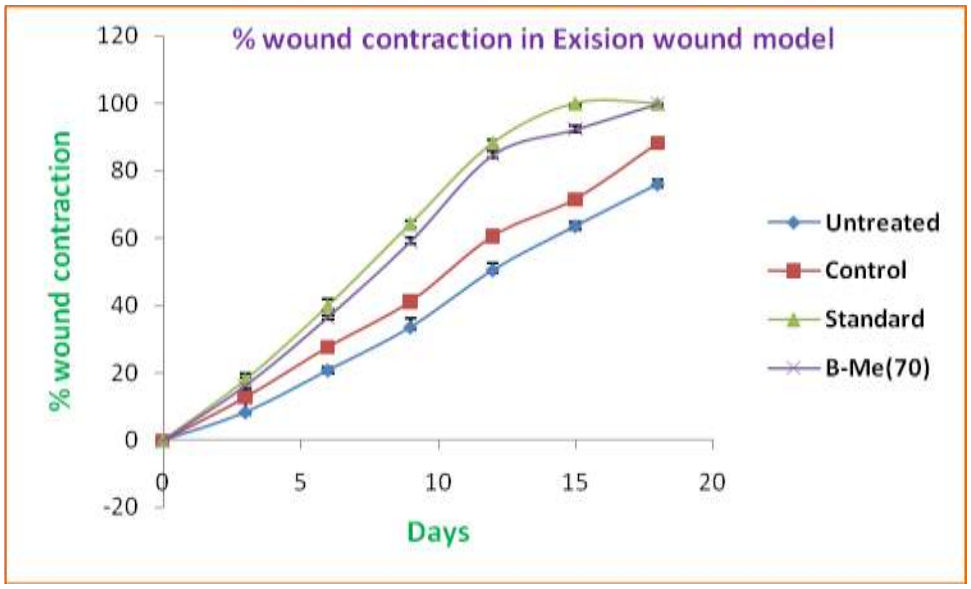

Fig.8. Graph showing wound healing profile.

CONCLUSION

Different parts (particularly leaves and stem bark) of *Grewia multiflora* Juss. (Malvaceae) are used by local vaidyas and tribal people for treatment of many diseases such as jaundice, diabetes, bone fracture, microbial infections, wound and worm infestation etc. Lliterature findings showed very less study about this plant. The defatted bark powder was extracted by soxhlation using Cm, Ac, Me, Aq. A portion of the residue after chloroform extraction was extracted with methanol and purified water mixture (70:30). Methanol(70) solvent was found to be more efficient towards extraction of major pharmaceutically active phytoconstituents. Methanol(70) extract [B-Me(70)] and methanol extract [B-Me] were selected after thorough study of presence of phytochemicals, content of flavonoids and phenolics for pharmacological potential study. Bark extracts were found to be safe for use as observed by toxicity study. B-Me(70) extract of *Grewia multiflora* Juss showed significant analgesic activity as proved by the methods studied. B-Me(70) might have inhibited the pain mechanism by blocking the nerve endings. The B-Me(70) extract of *Grewia multiflora* Juss showed significant anti-inflammatory activity ($p<0.01$) with inhibition of inflammation at 300mg/kg dose. The B-Me extract of *Grewia multiflora* Juss also showed moderately significant inhibition of inflammation at 300mg/kg dose but lesser than B-Me(70). The anti-inflammatory activity of the B-Me(70) extract may be attributed to the presence of different Phyto-constituents especially flavonoids. Flavonoids reduce the release of prostaglandin

minimizing tissue exaggeration. B-Me(70) extract of *Grewia multiflora* Juss at 100mg/mL concentration exhibited anthelmintic activity against adult earthworms *Pheretima posthuma* in short time causing paralysis(15.71minutes) and death(27.31 minute). It was observed that the ointment of B-Me(70) extract (15%) showed significant (p<0.01) ZOI against all the bacteria where as ointment of B-Me extract(15%) showed less significant (p<0.05) ZOI. The observed antimicrobial activity could be attributed to the phenol and flavonoid constituent of the extracts. The collagen synthesis is increased with developed tensile strength and thus completion of healing of wound occurred while treated with the ointment of B-Me(70) extract. Tannins and flavonoids present in extract exert the wound healing action.

STATISTICAL ANALYSIS:

All the data were presented as mean± SEM. The statistical differences were evaluated by ANOVA, followed by Student *t*-test. Statistical significance on comparison with the control were considered by $^{\#}p$<0.001 highly significant, $^{\wedge}p$<0.01 more significant and $^{*}p$<0.05 significant.

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ABBREVIATIONS

Cm : Chloroform
Me :Methanol
Ac : Acetone
Aq : Aqueous
FCR : Folin-Ciocalteu Reagent
GAE : Gallic acid equivalents
QE : Quercetin equivalent
SA : *Staphylococcus aureus*)
BL : *Bacillus licheniformis*

EC : *Escherichia coli*
ST : *Salmonella typhimurium*
SS : *Shigella sonnei*
VC : *Vibrio cholerae*
VA : *Vibrio alginolyticus*
SB : *Shigella boydii*
DMSO : Dimethyl sulfoxide
ZOI : Zone of inhibition

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