World Journal of Pharmaceutical ReseaRch

Volume 3, Issue 2, 2741-2752. Research Article ISSN 2277 – 7105

DESIGN AND BIOLOGICAL SCREENING OF SOME NOVEL FORMAZAN DERIVATIVES FROM SCHIFF BASES OF GALLIC ACID

Kumara Prasad S. A.*, Subrahmanyam E.V.S., . Shabaraya A.R.,

Srinivas College of Pharmacy, Valachil, Prangipete post, Mangalore - 574143, Karnataka, India.

Article Received on
07 January 2014
Revised on 25 January 2014,
Accepted on 24 February 2014

***Correspondence for Author**

Kumara Prasad S. A

Srinivas College of Pharmacy, Valachil, Prangipete post, Mangalore - Karnataka, India

ABSTRACT

A series of novel formazans were synthesized by multistep reaction starting from Gallic acid (3,4,5-trihydroxy benzoic acid). Gallic acid, after esterification, on reaction with hydrazine hydrate was converted into galloyl hydrazide. This intermediate compound underwent Schiff reaction with different aromatic aldehydes to yield ten Schiff bases. This Schiff bases on condensation with diazonium salts of various substituted anilines yielded formazan derivatives. All the compounds were obtained in good yield in the range of 60-80%. Melting points of the synthesized compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (MERCK, 60F) using chloroform: methanol (8:2) solvent system. The developed chromatographic plates were visualized under UV at 254nm. IR spectra were recorded using KBr on Josco FTIR model 8400 spectrophotometer, 1H NMR spectra in DMSO on a BRUKER FT-NMR instrument using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102 (DA-6000 mass Spectrometer) Data system using Argon (6KV.10MA) as the FAB gas. The Pharmacological screaning of all the synthesised compounds was performed to test their analgesic, antiinflammatory and anticonvulsant activity. Also the designed compounds were screened for Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli and* Antifungal activity against *Aspergillus Niger* in comparison with Ofloxacin and Fluconazole as standard to reveal the potency of synthesized derivatives. In accordance with the data obtained from Pharmacological and Antimicrobial screaning, all the synthesized Formazan derivatives have shown good Biological activity.

KEYWORDS Biologicall activity, Aromatic aldehyde, DMSO, Gallic acid, Formazan.

INTRODUCTION

Gallic acid (3,4,5-trihydroxybenzoic acid) is a polyhydroxyphenolic compound and Found in various natural products, like gallnuts, sumac, tea leaves, oak bark, green tea, apple-peels, grapes, strawberries, pineapples, bananas, lemons, and in red and white wine[1] and posses various biological activities these are antioxidant[2,3], antimelanogenic activity[4], antibacterial, antifungal and antiviral activities[5], Neuroprotective properties, antidiabetic activity[6], antimalarial[7], anti-carcinogenic[8], antimutagenic and anti-allergic[9], Anti-inflammatory activity[10], Induces apoptosis of tumor cells[11], Direct inhibition of several enzyme activities[12].

Also Formazans have been found to possess important medical applications. Numerous reports have been found regarding the formazan and it's derivatives for different pharmacological properties like, anti-inflammatory activity[13] was shown by derivatives like Quinazolino Formazans. Antiviral activity[14] was shown by 1,3,5 substituted phenyl formazans. Antimicrobial activity[15,16,17] was shown by 3,1,5 substituted sulphonamidophenyl formazans, 2,1,4 substituted Formazans and 1,3,5 substituted formazans. Anticonvulsant activity[18] was shown by 1,1,3 substituted phenyl formazans. On the basis of above reports we coupled diazonium salts with Schiff bases of Gallic acid to yield Formazans. And it proved that this combination resulted in a significant increase in Biological activities.

MATERIALS AND METHODS

All the chemicals used to synthesize the title compounds were of laboratory grade and purchased from S.D. Fine Chemicals and Sigma Aldrich. All the reactions were carried out under prescribed laboratory conditions. Melting points of the synthesized compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (MERCK, 60F) using chloroform: n-butanol (7:3) solvent system. The developed chromatographic plates were visualized under UV at 254nm. IR spectra were recorded using KBr on Josco FTIR model 8400 spectrophotometer, ^{1}H NMR spectra in DMSO on a BRUKER FT-NMR instrument using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102 (DA-6000 mass Spectrometer) data system using Argon (6KV.10MA) as the FAB gas.

1. Synthesis of propyl gallate (Propyl 3,4,5-trihydroxy benzoate)

In a round bottom flask 42g (0.246mol) of Gallic acid, 187ml (150g, 2.5mol) of propanol and 5g (2.7ml) of conc. Sulphuric acid was taken. The mixture was refluxed for 4 hours. Excess

of alcohol was distilled off on a water bath and allowed to cool. Poured it slowly and with stirring on to 200g of crushed ice. Added sufficient ammonia solution to render the resulting solution strongly alkaline. Extracted the mixture with five 25 ml portion of ether, dry the combined ethereal extracts over $MgSO_4$, removed the ether and distilled the residue under pressure.

2. Synthesis of Galloyl hydrazide (3,4,5-trihydroxy benzoyl hydrazide)

Propyl Gallate 21.2g. (0.1 mol) in 50ml ethanol and hydrazine hydrate 10ml (0.2 mol) were refluxed for 6 h. The excess of solvent was distilled off under reduced pressure using a vacuum pump. The cold residual mass was washed with distilled water, filtered and dried. The crude product obtained was recrystallised from methanol to yield 3,4,5-trihydroxy benzoyl hydrazide.

3. Synthesis of Schiff bases(N'-substituted benzylidene 3,4,5-trihydroxy benzoyl hydrazide)

2.26g. (0.01 mol) of Galloyl hydrazide and 0.01 mol of aromatic aldehyde(4-nitro benzaldehyde & salicyladehyde) was dissolved in 30ml of ethanol, followed by addition of 2 ml glacial acetic acid. The solution was refluxed for 6 hrs. Then cooled to room temperature and poured in to ice cold water. The solid product was collected through filtration and then dried in an oven at 80 ºC. The product was redissolved in ethanol for recrystallisation and then dried to give a product.

4. Synthesis of diazonium chloride solution:

0.02mol of aromatic amine (Aniline, PABA, Para amino phenol, Para nitro aniline) in 10 ml ml of glacial acetic acid and 6 ml of Conc. Hydrochloric acid was diazotized with 2 grams of sodium nitrite in 2ml water at 0-5^{o} C.

5. Synthesis of N-(substituted phenylamino)-N'-(3,4,5-trihydroxy benzamido) substituted benzamidine

The diazonium chloride solution was added to the solution of N'-substituted benzylidene 3,4,5-trihydroxy benzoyl hydrazide (0.1mol) in 10 ml of cold pyridine maintaining the temperature below 10°C. The reaction mixture was left overnight at room temperature. Thereafter it was poured into 250 ml of ice cold water with continuous stirring. The dark coloured solid mass which separated out was filtered, washed repeatedly with water and recrystallized from Petroleum ether: Benzene (80:20).

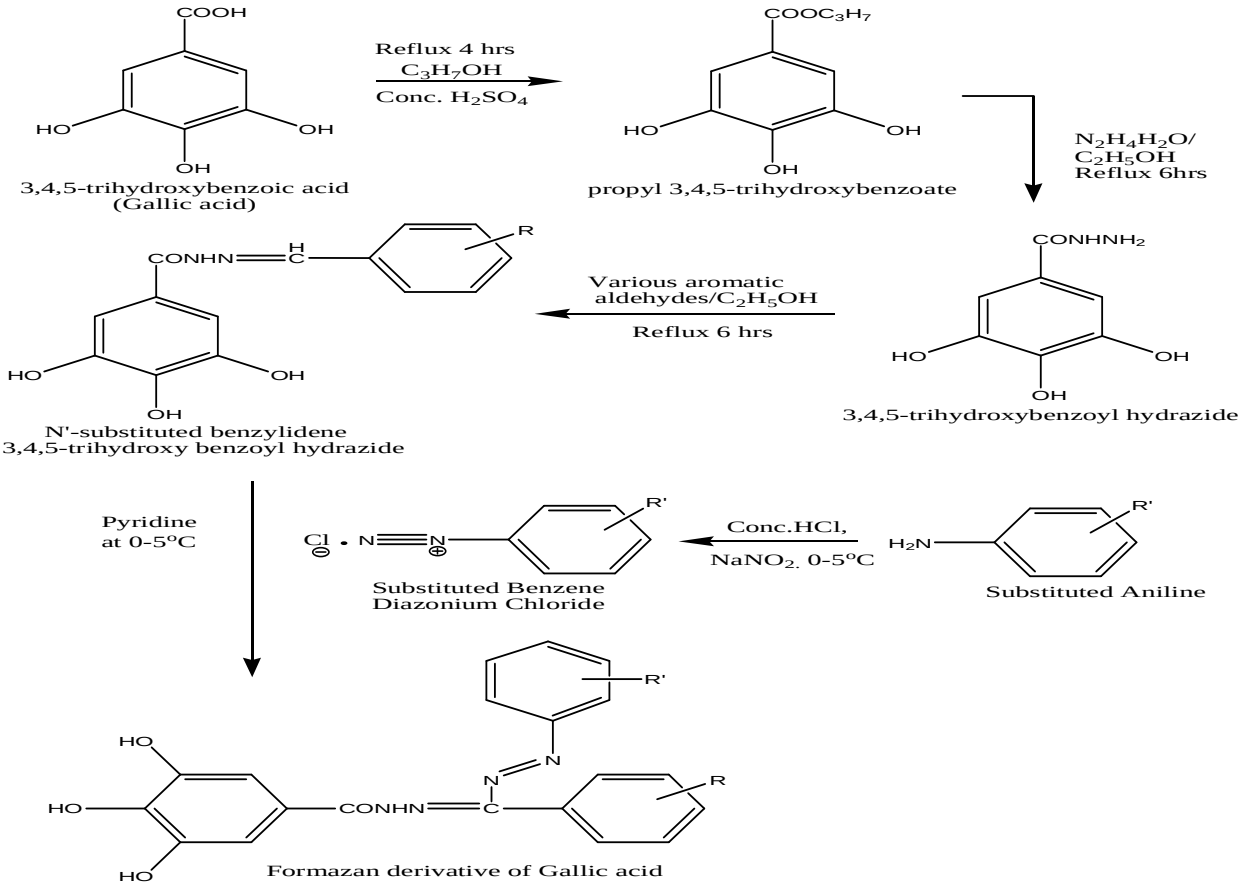

Figure-1 Scheme of Synthesis

Table-1 Physical constants data of synthesized compounds

Code	Compound Name	Mol. Formula	Mol. Weight	% Yield	Melting point
F-1	4- Nitro, N- phenylamino - N'-(3,4,5-trihydroxy benzamido) benzamidine	$C_{20}H_{15}N_5O_6$	421.10	75	105°C
F-2	4- Nitro, N-(4-carboxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine	$C_{21}H_{15}N_5O_8$	465.09	72	108°C
F-3	4- Nitro, N- (4-hydroxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine	$C_{20}H_{15}N_5O_7$	437.09	69	115°C
F-4	4- Nitro, N- (4-nitro phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine	$C_{20}H_{14}N_6O_8$	466.08	71	106°C
F-5	2 - hydroxy, N - phenylamino- N'- (3,4,5 - trihydroxy benzamido) benzamidine	$C_{20}H_{16}N_4O_5$	392.11	74	114°C
F-6	2 - hydroxy, N- (4-carboxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine	$C_{21}H_{16}N_4O_7$	436.10	78	124°C
F-7	2 - hydroxy, N- (4-hydroxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine	$C_{20}H_{16}N_4O_6$	408.10	70	112°C
F-8	2 - hydroxy, N- (4-nitro phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine	$C_{20}H_{15}N_5O_7$	437.09	73	118°C

SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

4- Nitro, N- phenylamino - N'-(3,4,5-trihydroxy benzamido) benzamidine(F1): IR (KBr in cm^{-1}): 1698.02 (C=N Str); 3370 (-N-H Str); 1204.33 (C-N Str); 1134.9 (C-O Phenolic); 3650.59 (O-H Str); 2965.98 (Ar.C-H Str); 1621.84 (Ar. C=C Str); 1698.02 (C=O Str); 1281.47 (-N =N Str); 1559 & 1387 (N=O Str nitro). ^{1}H NMR (DMSO, δ ppm): 5.035-5.051 (m, 3H, C-OH), 6.71 (s, 1H, NH), 7.94-8.26 (m, 4H, Nitro Ar.H), 6.82-7.36 (m, 7H, Ar.H). MS m/z, 420 [M^+].

4- Nitro, N-(4-carboxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine (F2): IR (KBr in cm^{-1}): 1628.59 (C=N Str); 3387.35 (-N-H Str); 1265.07 (C-N Str); 1212.04 (C-O Phenolic); 3611.29 & 3387.25 (O-H Str); 3198.36 (Ar.C-H Str); 1597 (Ar. C=C Str); 1628.41 & 1688.45 (C=O Str); 1319.07 (-N =N Str); 1541.81 & 1372.1 (N=O Str nitro). ^{1}H NMR (DMSO, δ ppm): 5.03 (m, 3H, C-OH), 7.04 (s, 1H, NH), 7.52-8.30 (m, 4H, Nitro Ar.H), 6.81-6.84 (m, 7H, Ar.H); 10.21 (m, 1H, COOH). MS m/z, 464 [M^+].

4- Nitro, N- (4-hydroxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine (F3): IR (KBr in cm^{-1}): 1693.19 (C=N Str); 3395.07 (-N-H Str); 1270.86 (C-N Str); 1211.08 (C-O Phenolic); 3611.59 (O-H Str); 3197.04 (Ar.C-H Str); 1596.77 (Ar. C=C Str); 1630.52 (C=O Str); 1321 (-N =N Str); 1539.88 & 1371.14 (N=O Str nitro). ^{1}H NMR (DMSO, δ ppm): 4.99-5.02 (m, 4H, C-OH), 6.72 (s, 1H, NH), 7.70-8.22 (m, 4H, Nitro Ar.H), 6.69-7.02 (m, 7H, Ar.H). MS m/z, 435 [M^+].

4- Nitro, N- (4-nitro phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine (F4): IR (KBr in cm^{-1}): 1687.41 (C=N Str); 3324.68 (-N-H Str); 1160.94 (C-N Str); 1034.68 (C-O Phenolic); 3640.95 (O-H Str); 3252.36 (Ar.C-H Str); 1588.09 (Ar. C=C Str); 1652.7 (C=O Str); 1034.62 (-N =N Str); 1530.24 & 1332.57 (N=O Str nitro). ^{1}H NMR (DMSO, δ ppm): 5.17-5.19 (m, 3H, C-OH), 7.04 (s, 1H, NH), 7.44-8.30 (m, 4H, Nitro Ar.H), 6.71-6.84 (m, 7H, Ar.H). MS m/z, 465 [M^+].

2 - hydroxy, N - phenylamino- N'- (3,4,5 - trihydroxy benzamido) benzamidine (F5): IR (KBr in cm^{-1}): 1692.23 (C=N Str); 3279.36 (-N-H Str); 1299.79 (C-N Str); 1236.15 (C-O Phenolic); 3626.48 (O-H Str); 3155.67 (Ar.C-H Str); 1611.23 (Ar. C=C Str); 1642.98 (C=O Str); 1159.57 (-N =N Str). ^{1}H NMR (DMSO, δ ppm): 4.96-4.99 (m, 4H, C-OH), 7.03 (s, 1H, NH), 6.69-7.26 (m, 11H, Ar.H). MS m/z, 391 [M^+].

2 - hydroxy, N- (4-carboxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine (F6): IR (KBr in cm^{-1}): 1692.23 (C=N Str); 3248.5 (-N-H Str); 1237.11 (C-N Str); 1237.11 (C-O Phenolic); 3646.73 (O-H Str); 3065.3 (Ar.C-H Str); 1607.38 (Ar. C=C Str); 1632.26 & 1692.23 (C=O Str); 1150.33 (-N =N Str). ^{1}H NMR (DMSO, δ ppm): 4.96-5.04 (m, 4H, C-OH), 7.03 (s, 1H, NH), 6.69-8.22 (m, 11H, Ar.H); 10.18 (s, 1H, COOH). MS m/z, 435 [M^+].

2 - hydroxy, N- (4-hydroxy phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine (F7): IR (KBr in cm^{-1}): 1664.41 (C=N Str); 3282.25 (-N-H Str); 1269.9 (C-N Str); 1105.01 (C-O Phenolic); 3632.73 (O-H Str); 3092.44 (Ar.C-H Str); 1586.87 (Ar. C=C Str); 1634.38 (C=O Str); 1150.33 (-N =N Str). ^{1}H NMR (DMSO, δ ppm): 5.17-5.19 (m, 5H, C-OH), 7.02 (s, 1H, NH), 6.70-7.45 (m, 10H, Ar.H). MS m/z, 407 [M^+].

2 - hydroxy, N- (4-nitro phenylamino) - N'-(3,4,5-trihydroxy benzamido) benzamidine (F8): IR (KBr in cm^{-1}): 1741.41 (C=N Str); 3281.29 (-N-H Str); 1269.9 (C-N Str); 1105.01 (C-O Phenolic); 3625.52 (O-H Str); 3121.48 (Ar.C-H Str); 1586.16 (Ar. C=C Str); 1634.38 (C=O Str); 1150.33 (-N =N Str); 1455.03 & 1327.75 (N=O Str nitro). ^{1}H NMR (DMSO, δ ppm): 5.01-5.05 (m, 4H, C-OH), 7.05 (s, 1H, NH), 6.71-8.36 (m, 10H, Ar.H). MS m/z, 435 [M^+].

BIOLOGICAL EVALUATION

Anticonvulsant Activity (Maximum Electroshock Method)

All the synthesized compounds were screened for their anticonvulsant activity using Electroconvulsiometer. Electro-convulsive shock, induce Hind Limb Tonic Extension (HLTE) in the animals. The electrical stimulus (50 mA; 50 Hz; 1-sec duration) was applied through ear-clip electrodes using a stimulator apparatus. Animals were divided into 10 groups of 3 each. First group served as control and animals were treated with DMSO. Another group of 3 mice were treated with reference or standard drug phenytoin (25mg/kg). Rest eight Groups of mice were pre-treated with test drug (50 mg/kg), and after 30 mins each group received the electroshock through ear-clip electrodes. The criterion for the anticonvulsant effect was considered the disappearance of HLTE within 10s after delivery of the electroshock. The results of determination of anticonvulsant activity are summarized in table No 2 with that of the standard drug.

Table 2 Anticonvulsant activity of the synthesized compounds

Sl. No.	Treatment	Duration time in sec (mean)	% protection
1	Control	15.17	00%
2	Std phenytoin	0.00	100%
3	F-1	7.54	50.29%
4	**F-2**	**6.82**	**55.04%**
5	F-3	8.13	46.40%
6	**F-4**	**6.26**	**58.73%**
7	F-5	11.06	27.09%
8	F-6	8.22	45.81%
9	F-7	9.56	36.98%
10	F-8	8.08	46.73%

Analgesic Activity (Eddy's Hot Plate Method)

Overnight fasted male albino mice were placed individually on a thermostatically controlled heated metal plate (55^{o}C) within a restraining perspex cylinder and the reaction time of each mouse was recorded. The mice showing initial reaction time of 10 sec or less were selected for this study and were divided into 10 groups (3 in each group). Group I served as control receiving DMSO, Group II received the standard drug pentazocine at the dose of 48mg/kg b.wt. Animals in Groups III to X were treated with the test drug at the dose of 50 mg/kg b.wt. Thereafter, the reaction time of each mouse was recorded at various intervals after the drug administration with a cut-off time of 15 sec. The increase in reaction time in drug-treated groups was compared with that of the control group. The results of determination of anlgesic activity are summarized in table No 3 with that of the standard drug.

Anti-inflammatory activity: (Carrageenan induced rat paw oedema inhibition method)

The rats were divided in to 10 groups of 3 animals each. Edema was induced by injecting 0.1 ml of carrageenan (1 % w/v) in saline solution into the sub-plantar region of the left hind paw of the rats, 1 hr. after oral administration of compounds at 50 mg/kg,. The control group received DMSO as vehicle. Diclofenac (100mg/kg) was used as standard drug. The average volume of right hind paw of each rat was measured at 0.5th,1st, 3rd, 5th h after the injection of carrageenan by using pleythysmograph. The results of determination of anti-inflammatory activity are summarized in table No 4 with that of the standard drug.

Table 3 Analgesic activity of the synthesized compounds

Sl.No	Treatment	Reaction time in minutes at					
		0 min.	30 Min.	60 Min.	90Min.	120 Min.	150 Min.
1	Ccontrol	3. 3.23	3. 3.35	2.2.47	2.2.14	2.2.51	2.2.54
2	Pentazocin	2.56	7.02	12.54	13.06	10.25	6.23
3	F-1	2.58	3.54	6.01	6.36	5.55	3.48
4	F-2	2.57	3.26	5.42	6.01	5.06	3.11
5	F-3	**3.05**	**4.56**	**7.15**	**7.59**	**6.23**	**4.15**
6	F-4	3.16	3.55	5.58	6.25	5.17	3.26
7	F-5	2.45	3.36	5.42	6.11	4.56	3.01
8	F-6	2.55	3.51	6.03	6.54	5.22	3.33
9	F-7	**3.11**	**5.46**	**9.47**	**9.48**	**7.41**	**5.14**
10	F-8	3.14	4.01	6.22	7.08	6.16	3.58

Anti-inflammatory activity

.

Table 4 Anti-inflammatory activity of the synthesized compounds:

Sl. No.	Treatment	Paw volume at different time interval after Carrageenan injection				
		0 h	0.5 h	1 h	3 h	5 h
1	CControl	0.05	0.15	0.40	0.43	0.49
2	S Diclofenac	0.05	0.07	0.13	0.20	0.11
3	F-1	0.06	0.14	0.21	0.28	0.18
4	F-2	0.05	0.10	0.20	0.29	0.21
5	F-3	**0.05**	**0.09**	**0.19**	**0.34**	**0.18**
6	F-4	0.06	0.10	0.19	0.31	0.17
7	F-5	0.06	0.10	0.20	0.26	0.20
8	F-6	0.05	0.12	0.19	0.27	0.19
9	F-7	**0.05**	**0.08**	**0.16**	**0.36**	**0.20**
10	F-8	0.06	0.09	0.23	0.33	0.22

Antimicrobial activity

The synthesised compounds were assayed for Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli and* Antifungal activity against *Aspergillus Niger* in comparison with Ofloxacin and Fluconazole as standard. Stock solutions of the synthesized compounds and standard drug used were prepared in DMSO and taken in the concentration of 100 µg/ml.

The Petri dishes were washed thoroughly and sterilized in hot air oven at 160^{o} C for one hour. 30 ml of sterile nutrient agar media for bacteria and potato dextrose agar media for fungi was poured in to sterile Petri dishes and allowed to solidify. The plates were incubated at 37^{o} C for 24 hours to check for sterility. The medium was seeded with the micro-organisms by pour plate method using sterile top agar (4 ml) containing 1 ml culture. The bores were made on the medium using sterile borer. Test compounds were dissolved in DMSO and 0.1 ml (100 µg/ml) of the different test compounds was added to the respective bores. 0.1 ml of Ofloxacin and fluconazole at a concentration of 100 µg/ml was taken as standard reference and 0.1 ml of DMSO was used as control. The Petri dishes were kept in refrigerator at 4^{o} C for 15 minutes, allowing diffusion to take place. The Petri plates were incubated at 37^{o} C for 24 hours for bacteria and 28^{o}C for 48 hours for fungus. Zone of inhibition were observed and measured using a scale. The results of determination of antimicrobial activity are summarized in table No 5 with that of the standard drug.

Table 5 Antmicrobial activities of the synthesized compounds

Sl. no.	Compound	S.aureus		E.coli		Aspargillus Niger	
		Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition
1	**F-1**	8.8	44	8.3	42	10	40.6
2	**F-2**	**12.7**	**63.5**	**11.2**	**57.1**	**15.3**	**62**
3	**F-3**	11.6	58	10.9	56	9.5	38.6
4	**F-4**	**12.2**	**61**	**11.8**	**60**	**15.1**	**61**
5	**F-5**	9.9	49.5	9.2	46.9	12.3	50
6	**F-6**	8.2	41	8.1	41	10.2	41
7	**F-7**	11.8	59	9.2	46.9	14.6	59.3
8	**F-8**	10.1	50.5	9.8	50	14.3	58.1
9	Ofloxacin	20	100	19.6	100	--	--
10	Fluconazole	--	--	--		24.6	100

RESULTS AND DISCUSSION

All the synthesized compounds were purified by successive recrystallization using ethanol. The purity of the synthesized compounds was checked by performing TLC. The structures of the synthesized compounds were determined on the basis of their FTIR and 1HNMR data and mass spectrum. The IR spectra of the synthesized compounds showed the presence of C=N stretching bands near 1665 cm^{-1}, C-N stretching bands near 1270 cm^{-1} and -N =N stretching frequencies 1150 cm^{-1} corresponding to Formazan compounds.

The pharmacological and antimicrobial screening for synthesised formazans was carried out.

The analgesic activity was carried out by eddys hot plate method, the anti inflammatory activity was carried out by carrageenan induced paw oedema method and anti epileptic activity by MES model. The standard drugs used were pentazocine diclofenac sodium and phenytoin for analgesic, anti inflammatory and anticonvulsant activity respectively. Screening for antimicrobial activity was done by cup plate metod by using Ofloxacin and Fluconazole as standard. All the Pharmacological and microbiological data are summarised in Table No- 2 to Table No-5.

In accordance with the data obtained from biological activity, all the synthesized Foamazans have shown good activity. Among these, compounds F-3 and F-7 having electron releasing groups on phenylamino ring has shown good analgesic and antiinflammatory activity. Compounds F-2 and F-4 with more number of electron withdrowing group has shown good anticonvulsant and antimicrobial activity.

CONCLUSION

In the present study, series of formazan derivatives from schff bases of gallic acid have been synthesized and confirmed through the spectral data. Further, they have been screened for various pharmacological activity studies like analgesic, anti inflammatory, anti epileptic by various methods. Also screened for antimicrobial activity by cup plate metod. It was concluded that these synthesized compounds have the potential of being useful in the treatment of such disorders for which they have been screened in the present study.

ACKNOWLEDGEMEN

The authors wish to thank Management of Srinivas College of Pharmacy, Valachil, Mangalore for the necessary facilities and encouragement. Also thanks to Indian Institute of Sciences, Bangalore for carrying out IR, HNMR and mass spectra.

REFERENCES

1. Sibylle Madlener , Illmer Christoph , Horvath Zsuzsanna, Saiko Philipp, Losert Annemarie, Herbacek Irene, Grusch Michael, Elford Howard L., Krupitza Georg, Bernhaus Astrid, Szekeres Monika Fritzer , Szekeres Thomas (2006) , “Gallic acid inhibits ribonucleotide reductase and cyclooxygenases in human HL-60 promyelocytic leukemia cells, *Cancer Letters*, 245, pp. 156 – 162.
2. Sroka, Z., Cisowski, W., (2003) “Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids” *Food and Chemical Toxicology*, 41, pp. 753–758.

3. Miyazawa T., (2000).“Absorption, metabolism and antioxidative effects of tea catechin in humans” *BioFactors*, 13(1-4), pp. 55-59.
4. Murase T., Kume N., Hase T., (1999). “Gallates inhibit cytokine-induced nuclear translocation of NfkappaB and expression of leukocyte adhesion molecules in vascular endothelial cells” *Arterioscler Thromb Vasc Biol.*, 19(6), Jun., pp. 1412-20.
5. Kubo I., Xiao P, Fujita K. (2001) “Antifungal activity of octyl gallate: structural criteria and mode of action” *Bioorganic & Medicinal Chemistry* Letters, 12; 11(3), Feb. pp. 347-50.
6. Ren Yulin , Klaus Himmeldirk, and Xiaozhuo Chen (2006) “Synthesis and Structure−Activity Relationship Study of Antidiabetic Penta-o-galloyl-d-glucopyranose and Its Analogues” *Journal of Medicinal Chemistry*, 49 (9), pp. 2829–2837.
7. Griffith, R., Chanphen, R., Scott, P. L. and Paul A. Keller (2002) “New Anti-Malarial Compounds from Database Searching” *Bioorganic & Medicinal Chemistry Letters,* 12, pp. 539–542.
8. Wang Y.C. and Bachrach U. (2002) “The specific anti-cancer activity of green tea (-)-pigallocatechin- 3-gallate (EGCG)” *Amino Acids*, 22, pp. 131–143.
9. Fukumoto, L. R., & Mazza, G. (2000) “Assessing antioxidant and prooxidant activities of phenolic compounds” *Journal of Agricultural Food Chemistry*, 48, pp. 3597–3604.
10. Nagai K., Jiang M. H., Hada J, Nagata T., Yajima Y., Yamamoto S, Nishizaki T., (2002) “(-) Epigallocatechin gallate protects against NO stress-induced neuronal damage after ischemia by acting as an anti-oxidant” *Brain Research*, 29, Nov. pp. 319-22.
11. Brusselmans K., Schrijver, E D., Walter Heyns, Guido Verhoeven, Johannes V. Swinnen (2003) “Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells” *International Journal of Cancer*, Volume 106 Issue 6, 26 Jun pp. 856 – 862.
12. Oku N., Matsukawa M., Yamakawa S., Asai T., Yahara S., Hashimoto F., Akizawa D & T (2003) “Inhibitory Effect of Green Tea Polyphenols on Membrane-Type 1 Matrix Metalloproteinase, (MT1-MMP)” *Biol. Pharm. Bull.* 26(9), pp. 1235-1238.
13. Kalsi R, Pande K, Bhall T, Barthwal JP, Gupta GP, and Parmar SS. Anti-inflammatory activity of Quinazolinoformazans. J pharm Sci 1990 Apr;79(4):317-20.
14. Pandey VK, Negi HS. Synthesis of 1-(2'-aryl-4'-oxo (3H) quinazolyl)-3-aryl-5-phenyl-formazans as potential anti-viral agents. Indian Drugs 1999;36(1):37-40.
15. Desal RM, Desai JM, and Shah VH. Synthesis and antimicrobial profile pf 1,3,4-oxadiazoles, sulphonamides, 5-imidazolinones, azomethines, 4-thiazolidinones, 2-

azetidinones and formazans and tetrazolium chlorides. Ind J Het Chem 1999 Apr-Jun;8:329-34.

16. Desai JM, and Shah VH. Synthesis and antimicrobial profile of 5-imidazolinones, sulphonamides, azomethines, 2-azetidinones and formazans derived from 2-amino- 3-cyno -5-(5'- chloro-3'- methyl-1'- phenylpyrazol -4'-ylvinyl)-7,7- 6,7-dihydrobenzo thiophenes. Ind J Chem 2003 Mar;42B:631-5.
17. Desai KG, and Desai KR. Synthesis of some novel pharmacologically active schiff bases using microwave method and their derivatives formazans by conventional method. Ind J Chem 2005 Oct;44B:2097-2101.
18. Archana, Srvastava VK, Kumar A. Synthesis of newer indolyl thiadiazoles and thiazolidinones and formazansas potent anticonvulsant agents. Ind J Pharm Sci 2003; 65(4):358-62.
19. Barsoum BN, Khella SK, Elwaby AHM, Abbas AA, Ibrahim YA. Evaluation of some new 14-and 15-crown-formazans as carriers in cesium ion selective electrodes. Talanta 1998;47:1215-22.
20. Tripathi KD. Essentials of Medical Pharmacology. 5th ed. New Delhi: Jypee publisher; 2003.