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Original Article

Isolation, Characterization and Assessment for Muscle Relaxant Activity of Novel Phytomolecule from *Galphimia glauca* Cav. Stems

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ABSTRACT

Objectives: *Galphimia glauca* Cav (Gg) is an evergreen medicinal herb found growing in Indian subcontinent and more in the Deccan plateau regions of south India. The objective of the current work is to isolate, characterize and explore the muscle relaxant activity of the novel isolated phytomolecule (BS-4) from *G. glauca* stems using *in vivo* models. **Methods:** The obtained bio-active fraction (methanol fraction) of the *G. glauca* stem methanol extract (GgSME) was subjected to column chromatography and Preparative thin layer chromatography (TLC) to isolate the bioactive phytomolecule. The novel isolated bioactive phytomolecule was coded as "BS-4", it is then characterized by R_f value, melting point, IR spectra, HPLC, Mass spectra, ^{1}H-NMR spectrum and ^{13}C NMR spectrum and evaluated for muscle relaxant activity by administering BS-4 in Swiss albino mice in one day to animal models like Rota rod test and Grip strengthening test. **Results:** The LD_{50} of BS-4 was > 2000 mg/kg. The mice were treated with BS-4 at 12.5, 25 and 50mg/kg doses. The BS-4 showed dose dependent significant ($P \leq 0.001$) effects on muscle relaxant activity in Rota rod test and Grip strengthening test in mice ($P \leq 0.001$). **Conclusion:** The current study results conclude that the isolated novel phytomolecule BS-4 has significant muscle relaxant effects.

Keywords: *Galphimia glauca* Clav, Column chromatography, Rota rod test, Grip strengthening test, Phytomolecule.

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INTRODUCTION

The importance of medicinal plants is recognized and documented well by research scholars since ancient history. Apart from the social benefits much attention has been given to the plants of medicinal significance. There is a worldwide resurgence in traditional and alternative systems of medicine resulting global herbal trade, which stands at US$ 120 billion and is expected to reach US $ 7 trillion by 2050. Most of the people in the developing nations believe in alternative system of medicine which uses medicinal plants for their primary health care. Due to the continuously increasing demand for medicinal plants, many herbal research institutes across the globe are engaged in research, documentation and developing databases on the medicinal plants helping the scientific community.[1]

The universe presently is facing challenges related to lifestyle and non-communicable diseases. Phytomedicine offer solutions to these challenges. Among the sources of medicines, the plants are the primary source for most of the lead molecules of therapeutic significance. Till date, many of the traditional plants whose therapeutic potential is not yet explored.

Galphimia glauca Cav.(Gg) is a shrub which grows up to 2-3 m height, it belongs to the family of Malpighiaceae.[2] This medicinal shrub is noticed growing across the globe. In India, it is found in all the states, specifically in Deccan plateau regions and in South India.

The plant is recognized as "*Calderona amarilla*" and "*Flor estrella.*" [3, 4] The ethyl acetate extract of Gg aerial parts were reported for its anti-asthmatic effects acting through inhibiting the Leukotriene D_4 induced muscle contraction.[5] Del-Rayao-Camacho *et al.* 2002 reported the isolation of Galphin-A, B and C, galphimidin, quercetin, sitosterol 3-O-β-d-glucoside and stigmasterol from Gg aerial parts.[6] Nader *et al.* 2006, disclosed the production of triterpenoids in the liquid cultivated hairy root of Gg[7]. Aguilar-Santamaria *et al.* 2007, disclosed the toxcological and cytotoxic actions of Gg aqueous, ethanol and methanol extracts[8]. Tortoriello *et al.* 2011, disclosed the structures of nor-seco-triterpene molecules isolated from methanol extract of Gg aerial parts[9]. Galphimine-A, B and C were disclosed by Cardoasa taketa. 2004.[10]

The present research on phytomedicine replaces the plant extracts with phytomolecules having a potential bioactivity, where effective phytomolecules come from medicinal plants.[11] The tea made from Gg yellow coloured leaves is used traditionally for lowering the fever, to relive coronary pain, soothing the nerves and emolliating injuries [12, 13]. It is a remedy for nervous excitement, pain and inflammation. [14-15]

To relate the traditional use, in our previous research we have proved that the Gg stem and leaf methanol extracts exhibited significant analgesic, anti-inflammatory, CNS depressant and muscle relaxant activities. [12,13, 16, 17] In the previous studies we have isolated two novel phytomolecules showing significant analgesic and CNS effects.[18, 19] Seeing the outcomes of previous work on Gg stem methanol extract in treating pain and inflammation, the current research was performed as an extension of earlier work at our lab. This study was mainly concentrated on muscle relaxant activity guided isolation and characterization of a novel molecule form Gg stems.

MATERIALS AND METHODS

Plant Material

The *Galphimia glauca* (Gg) was collected from the medicinal garden present in the Anurag University, Hyderabad. The stems were collected

in July 2019, dried and powdered. The plant was authenticated by Dr. E. Narsimha Murthy, Taxonomist, Satavahana University, Karimnagar, Telangana, India. A voucher copy is stored with the reference number No. 333, in the Department of Pharmacy, Anurag University.

Chemicals and drugs

The chemicals and Preparative TLC chromatography (# 350) were purchased from SD Fine Chemicals, Mumbai, India. Silica gel for Column chromatography (# 230-400) was purchased from Finar, India. TLC plates were purchased from Merck, Germany. Diazepam was purchased from Natco Pharmaceuticals, India.

Preparation of the extract

Gg stem powder (0.20 kg) was subjected to Soxhlet extraction using methanol (0.6 litres). The extract was collected, concentrated to dryness and stored. The yield obtained for Gg stem methanol extract (GgSME) was 0.040 Kg.

Animals

For the current study Swiss albino strain was used. The mice of 42 - 56 days old (22.5 ± 2.5 g) of either sex were employed for the study. Mice were acclimatized for ten days in the lab environment. The mice were maintained with nutrition, hygiene and care under conditions like temperature (22 ± 2ºC), light (fluorescent tube lights) and relative humidity (45-55%). Twelve hours each of darkness and light was maintained with controlled noise ≤ 65 decibels. The studies were performed using six mice of either sex in each group. The study protocol was approved by the Institutional Animal Ethics Committee of the institute (IAEC), School of Pharmacy, Anurag University (the protocol number: I/IAEC/LCP/032/2018/19).

Acute Toxicity Studies

According to The Organization for Economic Co-operation and Development (OECD) guidelines, 423-2d, acute oral toxicity studies were conducted.[20]

Fractionations of Gg stem methanol extract

In our earlier work the Gg stem methanol extract exhibited significant muscle relaxant properties (Baba Shankar *et al*. 2016).[18] Hence, in this study 0.040 Kg of Gg stem methanol extract was dissolved in 0.085 litre of methanol and fractionated with 0.5 litres each of n-hexane, chloroform, ethyl acetate and methanol. The obtained fractions [Gg n-hexane fraction (GgH), Gg chloroform fraction (GgC), Gg ethyl acetate fraction (GgE) and Gg methanol fraction (GgM)] were concentrated and the yield was noted.

Phytochemical screening for the Gg stem methanol extracts fractions and BS-4

The Phytochemical screening was done to disclose the nature of phyto-constituents present in GgH, GgC, GgE and GgM fractions obtained from the Gg stem methanol extract and the isolated phytomolecule BS-4.[21] The methanol fraction (GgM) explored the nature of phyto-constituents such as Flavonoids, Saponins, Steroids & Terpenoids, Tannins and Phenolic compounds whereas the GgH, GgC, and GgE fractions showed negative results. The novel isolated phytomolecule BS-4 belongs to terpenoid.

Separation of phytoconstituents and characterization
Preparation of sample for separation

About 0.016 Kg of the Gg methanol fraction (GgM) is dissolved in equal volumes of methanol and water (250 ml + 250 ml), extracted (1:1 ratio) with ethyl acetate twice. The ethyl acetate fractions were pooled and concentrated to 75 ml volume and extracted with hexane in the ratio of 1:5. The hexane insoluble was separated and concentrated to get in powdered form. The powder is then dissolved in a 200 ml of ethyl acetate. 60 grams of silica powder was added to ethyl acetate solution to coat its surface. It was then subjected to vacuum evaporation using Heidolph rotary evaporator, dried and stored in vacuum desiccator. The silica powder was activated at 110°C before using in the column.

Column chromatography

The column is a borosilicate column, of about 80 cm length with an inside diameter of 3 cm. The Silica gel (230-400 µm) was used for this study.

Procedure

The Column employed was cleaned, dried and rinsed with chloroform before using. A cotton piece was inserted in the column at nozzle tip, which aids in filtration. Silica gel (150 grams) was used to make a slurry using chloroform and used for packing. The excess of solvent was drained out. The surface coated silica powder was loaded and a cotton piece was placed above it and the column was eluted with chloroform initially (300 ml), followed by solvents with changing polarities. 250 ml of ethyl acetate in chloroform was used for this purpose in varying percentages (0%, 10%, 20%, 30%, 40%, 50%, 52.5%, 55%, 57.5%, 60%, 62.5%, 65%, 67.5%, 70%, 72.5% 75%, 77.5%, 80%, 90%, and 100%). Individual fractions (25 ml) were collected and labelled.

TLC Studies

All the fractions collected were concentrated to 10 ml and subjected to thin layer chromatographic studies (TLC) using ethyl acetate: chloroform (80:20) as solvent system. The separated compounds were visualized with 10% of sulphuric acid in methanol. The identical fractions were pooled and labelled.

Preparative TLC chromatography

The combined fractions of column chromatography (13-27) were used for separation of phytoconstituents. The slurry was prepared by mixing 1.5 to 2.5 parts of distilled water to 1 part of silica gel and stirred perfectly. The slurry was used for coating the glass plates by pouring method'. Twelve glass plates (2 x 4 inch) were arranged in a row. The slurry was poured at the centre of glass plate and then distributed all over the surface. After leaving it for 15 min, the plates were air dried for 45 min. The plates were then activated in hot air oven at 110°C for of 2 hrs. The sample was loaded and developed in glass chamber using 10% methanol in chloroform as mobile phase. The separated compounds were cut, scrapped and collected.

Characterization of the novel isolated phytomolecule (BS-4)

The isolated phytomolecule was coded as "BS-4" The BS-4 was characterized by its R_f value, phytochemical screening, melting point, IR spectral data, HPLC, ^{1}H-NMR spectra, ^{13}C NMR spectra and Mass spectral studies. The BS-4 was soluble in DMSO-D6 and NMR studies were performed using JEOL USA Spectrophotometer (JNM-ECZ500R/S1).

Pharmacological studies on the novel isolated phytomolecule (BS-4)

Studies were conducted to assess the *in vivo* muscle relaxant activity for the isolated phytomolecule (BS-4) to explore its pharmacological significance. The dose range of 12.5, 25 and 50 mg/kg b.w was administered orally for assessing the studies.

Grouping of animals for muscle relaxant studies

The below mentioned grouping procedure is adopted for investigating muscle relaxant activity. The Swiss albino mice employed for the study were grouped separately (Group I-V). The mice were treated with 12.5 mg/kg (low dose), 25 mg/kg (medium dose) and 50 mg/kg (high dose) respectively.

Group-I: Negative control, treated with distilled water [10 ml/kg, per os (p.o)].

Group II: Positive control group, treated with diazepam [1 mg/kg, intraperitoneally (i.p)].

Group III-V was treated with BS-4 [12.5, 25 and 50 mg/kg, respectively, per os (p.o)].

Muscle coordination test

Grip strength test

This procedure was reported by Boissier and Simon (1960).[22] Mice were screened initially by placing them on a steel rod, which is fixed to a stand at a height of 0.5 meter. Mice which failed to remain hanging less than sixty seconds were rejected. The mice (Group1-V) fasted overnight were used for this study. The control group (Group-I) mice received water. After 1 hour post treatment with BS-4 as cited above in grouping of animals for muscle relaxant studies and intraperitoneal administration with diazepam (1 mg/kg), mice were again tested. A reduction in time for all the groups (Group II-V) is correlated with group-I as an estimate of muscle relaxant activity.

Rota rod test

This test was reported by Dunham and Miya (1957) to study effect of drugs on motor coordination.[23] The test was performed on the Rota rod apparatus which is separated into four individual compartments (V. J, instruments, Maharashtra). Animals fasted overnight were used for this study. The Group-I animals received water. After 1 hour post treatment with BS-4 as cited above in grouping of animals for muscle relaxant studies and intraperitoneal administration with diazepam (1 mg/kg), mice were placed on the rod, revolving at 24 rpm speed and the time of fall from the revolving rod is noted for each groups at 30 min, 60 min and 90 min respectively. The time of fall for all the groups (Group II-V) is correlated with a control group (Group I) as an estimate of muscle relaxant activity.

Statistical analysis

Numerical data was expressed as mean ± SEM (standard error of mean). Statistical analysis were performed with one-way analysis of variance (ANOVA), followed by Tukeys' multiple comparison test to compare results obtained for all tested groups with each other. $P \leq 0.05$ was considered to be statistically significant. The statistical analysis was carried out with Graph Pad Prism 5.0 software.

RESULTS

Acute toxicity studies

The Gg stem methanol extract, GgM and BS-4 did not exhibit any toxic symptoms and also Mortality in the range of 5, 50, 300 and 2000 mg/kg in mice during the 14 days of study. Hence it can be classified as category 5 according to OECD-423, guidelines. Therefore the Gg stem methanol extract, GgM and BS-4 were found to be non-toxic to mice. Based on the results and the yield of the BS-4, the doses, 12 mg/kg (low dose), 25 mg/kg (moderate dose) and 50 mg/kg (high dose) were chosen to study the biological activity.

Fractionations of Gg stem methanol extract (GgSME)

The active extract, Gg stem methanol extract (GgSME) was fractionated using n-hexane, chloroform, ethyl acetate and methanol. The yield obtained for n-hexane fraction (GgH), chloroform fraction (GgC), ethyl acetate fraction (GgE) and methanol fraction (GgM) fractions are 5.8%, 13.4%, 17% and 59.1% respectively.

Separation of phytoconstituents and characterization
Column chromatography

The bio-active fraction (GgM) was subjected to column chromatography. All fractions collected in 25 ml volume and labelled for their identification.

TLC Studies

All the fractions of 25 ml volume were collected and subjected to TLC studies. From the results, the fractions containing phytoconstituents with identical R_f values were grouped and concentrated to dryness (Fraction No: 13-27).

Preparative thin layer chromatography (TLC):

The Preparative TLC was performed using column fractions (13-27) to separate phytomolecules. The separated fractions (1-4) were cut and collected separately from glass plates labelled A-J. The fraction cut 3 containing individual separated phytomolecule was boiled with 500 ml of methanol in RBF and vacuum filtered. The filtrate was concentrated and subjected to hexane treatment. The hexane insoluble portion was concentrated to obtain novel isolated phytomolecule (BS-4). The yield obtained was 740 mg. The TLC of BS-4 was performed in solvent system 10% methanol in chloroform.

Characterization of the novel isolated phytomolecule (BS-4)

The novel isolated phytomolecule belongs to terpenoids. The melting point is 101.1°C-108.2°C. The R_f value was 0.3. The IR spectra shown in Figure 1 revealed the presence of -OH group, aliphatic -CH stretching vibrations, the ester carbonyl group, C-O-C stretching and C-O stretching. The mass spectra exhibited base peak, molecular ion peak and M+1 peak were at 663. The ^{1}H-NMR spectrum disclosed the aliphatic group in range from 0.7 to 2.8 ppm. The ^{13}C NMR spectra disclosed 113.833 (C-1), 107.905 (C-2), 100.109 (C-3) and 90.930 (C-4). The HPLC study revealed, that the major HPLC peak has shown retention time (tR) of 2.397 min with 100% purity. The Figure 2 & 3 represents the HPLC peak & HPLC purity index of BS-4. The UV λ_{max} was 299 nm.

Pharmacological studies of the novel isolated molecule (BS-4)
Grip strength test

The results are illustrated in Figure-4. The BS-4 at 50 mg/kg exhibited a significant dose dependent activity ($P \leq 0.01$) on comparison of the high dose with respective to low dose, which was proved by the in competence of the mice to hang on to a metal wire. In comparison of BS-4 with the control group and standard drug diazepam the percentage of mice loosing their grip exhibited a significant activity ($P \leq 0.001$).

Rota rod test

The study results are illustrated in Figure-5. There was a significant ($P < 0.001$) decrease in the span of time spent by the mice on revolving rod with BS-4 when correlated with control group and standard drug diazepam. The BS-4 at 50 mg/kg exhibited a significant dose dependent activity ($P \leq 0.01$) on comparison of the high dose with respective to low dose.

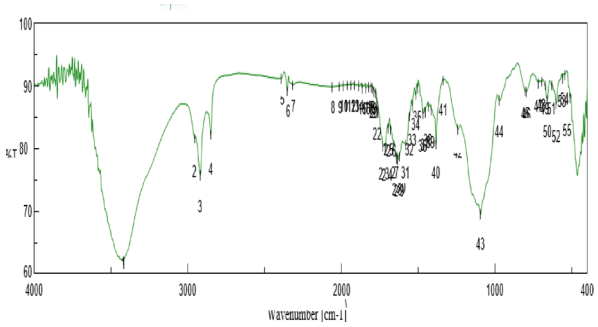

Figure 1: IR Spectra of Novel Phytomolecule BS-4

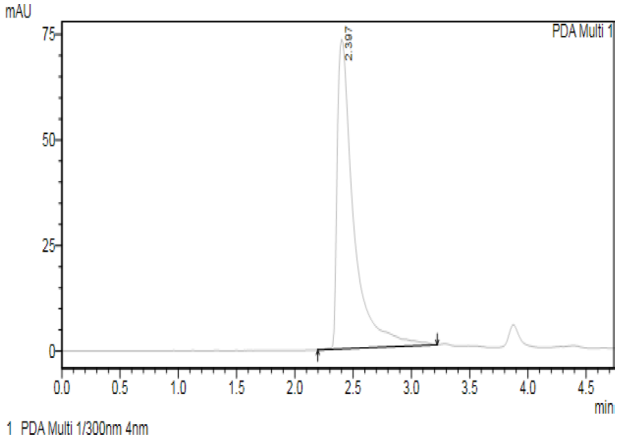

Figure 2: HPLC peak of Novel Phytomolecule BS-4. Peak Retention Time 2.397

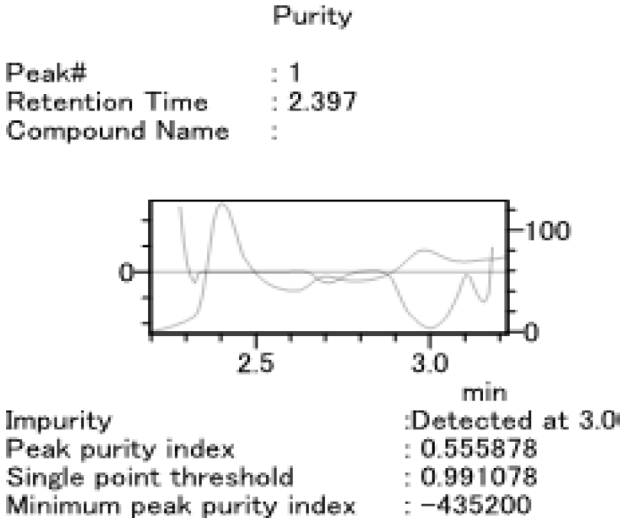

Figure 3: HPLC peak purity index of Novel Phytomolecule BS-4. Peak Retention Time 2.397

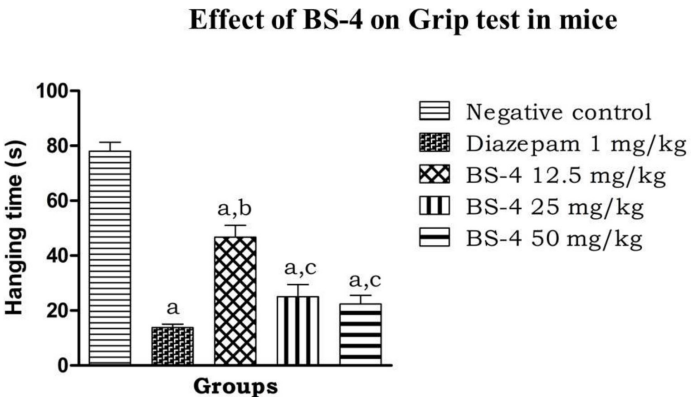

Figure 4 : Effect of BS-4 on Muscle relaxant activity-Grip test in mice.
Values are expressed as Mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests and is represented by a symbol.
[a]*P* ≤ 0.001 indicates comparison with group I
[b]*P* ≤ 0.001 indicates comparison with group II
[c]*P* < 0.001 indicates the dose dependent activity on comparison of the high does with respective low dose of the BS-4.

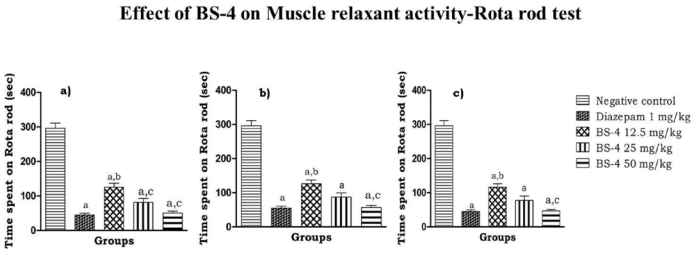

Figure 5 : Effect of BS-4 on Muscle relaxant activity-Rota rod test.
Time spent (s) by the mice on rota rod a) after 30 min; b) after 60 min; c) after 90 min
Values are expressed as Mean ± SEM.; n = 6; the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests and is represented by a symbol.
[a]*P* ≤ 0.001 indicates comparison with group I
[b]*P* ≤ 0.001 indicates comparison with group II
[c]*P* < 0.001 indicates the dose dependent activity on comparison of the high does with respective low dose of the BS-4.

DISCUSSION

Many of the bioactive molecules have come from plants. It is a need to put efforts to tap the real potential of natural sources of medicines. The Gg employed for this study is traditionally reported to treat conditions like fear, anxiety, stress and phobia. It also helps in women in labor, to lower the fever, to calm the patient and to relief from coronary pain. It is an emollient for injuries and for soothing the nerves.[24] The significance of this work is to isolate, characterize and evaluate the muscle relaxant activity of the bio-active phytomolecule from the Gg stems.

In our previous research work carried out on Gg stem methanol extract (GgSME), it was found that stem extract exhibited significant muscle relaxant activity.[17] Based on the results the active extract, Gg stem methanol extract (GgSME) was subjected to fractionation using solvents of different polarities. The fractions n-hexane (GgH), chloroform (GgC), ethyl acetate (GgE) and methanol (GgM) were subjected to phytochemical screening to explore the active fraction (GgM) that can be used for the isolation of bioactive phytomolecule. The GgM (methanol fraction) showed the presence of important phytomolecules like, tannins and phenolic compounds, steroids and terpenoids, saponins and flavonoids than the remaining fractions GgH, GgC and GgE.

The active fraction GgM was subjected to column chromatography. The solvent chloroform and ethyl acetate with changing concentrations from 100:0 to 0:100 were employed. All the collected fractions were subjected to TLC studies. From the TLC results, the identical fraction 13-27 containing compounds with identical Rf values were pooled and then subjected to preparative TLC studies using mobile phase 10% methanol in chloroform to separate the bio-active phytomolecule. The separated phytomolecule was treated with hexane. The hexane insoluble was concentrated to get novel phytomolecule (BS-4).

The novel isolated phytomolecule belongs to terpenoids. The melting point is 101.1°C-108.2°C. The R_f value was 0.3. The IR spectra revealed the presence of -OH group, aliphatic -CH stretching vibrations, the ester carbonyl group, C-O-C stretching and C-O stretching. The mass spectra exhibited base peak, molecular ion peak and M+1 peak were at 663. The ^{1}H-NMR spectrum disclosed the aliphatic group in range from 0.7 to 2.8 ppm. The ^{13}C NMR spectra disclosed 113.833 (C-1), 107.905 (C-2), 100.109 (C-3) and 90.930 (C-4). The HPLC study revealed, that the major HPLC peak has shown retention time (tR) of 2.397 min with 100% purity. The UV λ_{max} was 299nm.

The models to assess muscle relaxant activity include rota rod test and grip strength test.[23] The grip test is carried out to explore the muscle strength and neuromuscular activity, whereas rota rod experiment is carried out to check motor coordination in animals [25, 26]. The mouse which remained on the revolving rod, for a short span of time suggests the muscle relaxant effect. Abundant CNS drugs which were depressants, respond well to this test [27, 28]. In rota rod test, the BS-4 treated mice exhibited significant ($P < 0.001$) activity. Whereas the hanging time of mice was decreased significantly ($P < 0.001$) in comparison to control group. The diazepam exhibits muscle relaxation, acting on $GABA_A$ receptors involving α2 and α3 subunits, enhancing the potency of GABA to increase the chloride conductance causing muscle relaxant effect.[29,30,31] The BS-4 may act similarly to that of diazepam. The results suggest that, BS-4 was more potent for muscle relaxant effects

CONCLUSION

The novel isolated phytomolecule (BS-4) belongs to terpenoid. It exhibited significant muscle relaxant effects. This study facilitates the exchange of research and supports clinical use. The future studies will be focused for spectral data like 2-D NMR (HSQC, HMBC, COSY, NOESY etc.).

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CONFLICT OF INTEREST

The authors declare no Conflict of interest.

ABBREVIATIONS

Gg: Galphimia glauca; **GgSME:** Galphimia glauca stem methanolic extract; **GgH:** G. glauca n-hexane fraction; **GgC:** G. glauca chloroform fraction; **GgEA:** G. glauca ethyl acetate fraction; **GgM:** G. glauca methanol fraction; **IAEC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experimental Animals; **OECD:** The Organization for Economic Co-operation and Development; **WHO:** World Health Organization; **b.w:** Body weight; **i.p:** Intraperitoneal; **p.o:** per oral; **^{1}H-NMR:** Proton nuclear magnetic resonance; **13C NMR:** Carbon-13 Nuclear magnetic resonance; **AGI:** Anurag Group of Institutions; **BS-4:** Novel Isolated Phytomolecule.

REFERENCES

1. Veeresham C. Natural products derived from plants as a source of drugs. Journal of Advanced Pharmaceutical Technology and Research. 2012;3(4):200-1. DOI: 10.4103/2231-4040.104709.
2. Anderson C. Revision of *Galphimia* (Malpighiaceae). Contr Univ Michigan Herb. 2007;25:1-82.
3. Baba SRG, Srisailam K, Uma MRV. Standardization and elemental analysis of *Galphimia glauca* leaf and stem parts employing SEM-EDAX. Int J Pharm Bio Scien. 2016;6:54-61.
4. Tortoriello J, Maribel HR, Cortazar MG, Ferrer EJ, Zamilpa A, Alvar L, *et al*. Anxiolytic effects of natural galphimines from *Galphimia glauca* and their chemical derivatives. J Nat Prod. 2006;69(1):59-61. DOI: 10.1021/np050305x
5. Compos MG, Toxqui E, Tortoriello J, Oropeza MV, Ponce H, Vargas HM, *et al*. *Galphimia glauca* organic fraction antagonizes LTD(4)- induced contraction in guinea pig airways. J Ethnopharmacol. 2001;74(1):7-15. DOI: 10.1016/s0378-8741(00)00322-6
6. Maria DRC, Phillipson JD, Croft SL, Marley D, Kirby GC, Warhurst DC. Assessment of the anti-protozoal activity of *Galphimia glauca* and the Isolation of New Nor-secofriedelanes and nor-friedelanes. J Nat Prod. 2002;65(10):1457-61.
7. Nader BL, Taketa AT, Pereda MR, Villarreal ML. Production of triterpenoids in liquid cultivated hairy root of *Galphimia glauca*. Plant Med. 2006;72(9):842-4. DOI: 10.1055/s-2006-931601
8. Aguilar-Santamaria L, Ramirez G, Herrera-Arellano A, Zamilpa A, Jimenez JE, Alonso-Cortes D, *et al*. Toxicological and cytotoxic evaluation of standardized extracts of *Galphimia glauca*. J Ethnopharmacol. 2007;109(1):35-40. DOI: 10.1016/j.jep.2006.06.013
9. Tortoriello J, Herrera-Arellano A, Herrera-Ruiz ML, Zamilpa A, Gonzalez-Cortazar M, Jimenez-Ferrer JE. New Anxiolytic Phytopharmaceutical Elaborated with the Standardized Extract of *Galphimia glauca*, Anxiety Disorders. InTech; 2011;185-202. Available from: http://www.intechopen.com/books/anxiety-disorders/new- anxiolytic-phytopharmaceutical-elaborated-with-the-standardized-extract-of-galphimia-glauca
10. Cardoso TAT, Lozada-Lechuga J, Fragoso-Serrano M, Villarreal ML, Pereda-Miranda R. Isolation of nor-secofriedelanes from the sedative extracts of *Galphimia glauca*. J Nat Prod. 2004;67(4):644-9.
11. Pawar HA. Recent investigations in Phytopharmacology: An Overview. The Pharma Innovation Journal. 2013;2(4 Part A):28.
12. Baba Shankar rao G, Srisailam K, Uma MRV. Assessment of Anti-nociceptive and anti-inflammatory activities of *Galphimia glauca* stem methanol extract on noxious provocation induced pain and inflammation in *in-vivo* models. J Chem Pharm Res. 2016;28(8):1815-9.
13. Baba SRG, Srisailam K, Uma MRV. *In-vivo* evaluation of noxious stimulus induced pain and inflammation in mice and rats using methanol extract of *Galphimia glauca* leaf. Asian J Chem. 2016;28(8):1815-9. doi.org/10.14233/ajchem.2016.19839
14. Estrada E. Jardı´n Bota´nico de Plantas Medicinales "Maximino Martı´nez". Me´xico Universidad Auto´noma de Cha-pingo, Departamento de Fitote´cnia. 1985;15.
15. Tortoriello J, Lozoya X. Effect of *Galphimia glauca* methanolic extract on neuropharmacological tests. Planta Medica. 1992;58(03):234-6.
16. Baba Shankar rao G, Srisailam K, Uma Maheswara rao V. CNS Depressant effects and muscle relaxant activity of *Galphimia glauca* leaf methanol extract. Int. J. PharmTech Research. 2016;9(6):230-40.
17. Baba SRG, Srisailam K, Uma MRV. *In vivo* study on Depressant effects and Muscle coordination activity of *Galphimia glauca* stem methanol extract. Pharmacognosy Res. 2016;8(4):219-25. DOI: 10.4103/0974-8490.188878
18. Baba SRG, Srisailam K, Uma MRV. Isolation, Characterization and assessment for Central Nervous System Effects of Novel Phytomolecule from *Galphimia glauca* Cav. Stems. Asian Journal of Chemistry. 2019;31(6):1230-6. doi.org/10.14233/ajchem.2019.21748
19. Baba SRG, Srisailam K, Uma MRV. Innovative Research on Isolation, Characterization and Identification of Bioactivity in the Isolated Constituent from Methanol Extract of *Galphimia glauca* Cav. Stems. Pharmacognosy Res. 2018;10(3):265-74. DOI: 10.4103/pr.pr_151_17
20. OECD Guidelines 423. OECD guideline for testing of chemicals. Acute Oral Toxicity- Acute Toxic Class Method. 2001.
21. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, 21st edition. Pune: Niraliprakashan. 2002;38-161.
22. Boissier JR, Simpon P. L'utilisation du test de la traction, pour l' etude des psycholeptiques. Therapie. 1960;15:1170-4.
23. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharmaceut Assoc. 1957;46:208-10. DOI: 10.1002/jps.3030460322
24. Tortoriello X, Lozoya X. Effect of *Galphimia glauca* methanolic extract on neuropharmacological tests. Planta Medica. 1992;58(03):234-6. DOI: 10.1055/s-2006-961442
25. Adzu S, Amos S, Dzarma CW, Gamaniel K. Effects of Zizypus spina Christi wild aqueous extract on the central nervous system in mice. J Ethnopharmacol. 2002;79(1):13-6. DOI: 10.1016/s0378-8741(01)00348-8
26. Wei X, Yang J, Wang J, Wu C. Anxiolytic effects of saponins from Panax quinquefolium in mice. J Ethnopharmacol. 2007;111(3):613-8. DOI: 10.1016/j.jep.2007.01.009
27. Yemitan OK, Adeyemi OO. CNS depressant activity of *Lecaniodiscus cupanioides*. Fitoterapia. 2005;76(5):412-8. DOI: 10.1016/j.fitote.2005.02.010
28. Ashutosh Kar. Medicinal chemistry, 5th ed. New Delhi: New Age International (P) Limited. 2012;170-98; 225-52.
29. Thomas L, Lemke ID, Williams VF, Roche IS, William Z. Foye's Principles of Medicinal Chemistry, 7th ed. New Delhi: Wolters Kluwer (India). 2013;438-699.
30. Bennett PN, Brown MJ, Sharma P. Clinical pharmacology, 11th ed. China: Elsevier. 2012;240-94.
31. Vogel HG. Drug discovery and evaluation Pharmacological assays, 2nd ed. Berlin: Springer. 2002;390-493.

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