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Research Article

A New Triterpenoid Saponin from Tubers of Gloriosa superba Linn

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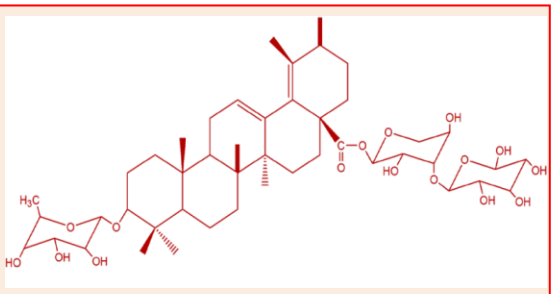
Abstract

This paper deals with the isolation and structural elucidation of a new compound (A), identified as 3β-O-α-L-rhamnopyranosyl-urs-12,18(19)-dien-28-oic acid-28-O-β-D-glucopyranosyl(1→3)-O-β-D xylopyranosyl ester along with two known compounds Taraxerol (B) and Friedelin (C) from the methanolic fraction of the tubers of the plant *Gloriosa superba* Linn. The isolated compounds so obtained from the methanolic fraction of the tubers of the plant were characterized and identified by various colour reactions, chemical degradations and spectral analysis. Plant extract was also utilized for Behaviour Despair Study in animal model by making use of Forced Swim Test and Tail Suspension Test; these tests reported antidepressant activity of the plant extract.



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Introduction

Gloriosa superba Linn.[1-4] belongs to Liliaceae family. It is commonly known as 'Kalihari' in Hindi. It is naturally found throughout tropical India, ascending up to 7000 ft. on the hills. It has a wide variety of uses, especially in traditional medicine; its leaves are used to treat infection of round worm, tape worm, liver fluke and filaria. Its roots are topically applied to treat arthritic conditions, swellings of the joints, sprain and dislocations. Its tubers are also used as an agent to promote labour pain or to bring about abortions. Earlier workers[5-8] has reported various constituents from this plant. This paper reports the isolation and structural elucidation of a new compound (A) identified as 3β-O-α-L-rhamnopyranosyl-urs-12,18(19)-dien-28-oic acid-28-O-β-D-glucopyranosyl (1→3)-O-β-D xylopyranosyl ester along with two known compounds Taraxerol (B) and Friedelin (C) from the methanolic fraction of the tubers of the plant. Plant extract was also utilized for Behaviour Despair Study in animal model by making use of Forced Swim Test and Tail Suspension Test; these tests reported antidepressant activity of the plant.

Experimental

General experimental procedure

All the melting points were determined on a thermoelectrical melting point apparatus and are uncorrected. The IR spectra were recorded in KBr disc on Perkin Elmer Spectrum RXI (4000-4500 cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were run at 300 MHz and 75MHz respectively on Bruker DRX NMR Spectrometers using TMS as internal standard and Pyridine-d_5 as solvent. Mass spectra run on Jeol SX-102 FAB Mass Spectrometer.

Plant material

Tubers of toxic plant *Gloriosa superba* Linn., were procured from in and around Saugor region and were taxonomically authenticated by Taxonomist Department of Botany, Dr. H.S. Gour Central University, Saugor

(M.P.) India. A voucher specimen (AY-1001GS) has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour Central University, Saugor (M.P.).

Extraction and Isolation

Air-dried and powdered tubers (4.5 kg) of the plant were extracted with 95% Ethanol (60-80°C) in a Soxhlet apparatus for one week. The total ethanolic extract was concentrated and successively partitioned with petroleum ether (40-60%), $CHCl_3$, $CH_3COOC_2H_5$, CH_3COCH_3 and CH_3OH. The methanol soluble fraction of the plant was concentrated under reduced pressure to give a light brown viscous mass (3.15 gm). It gave three spots on TLC examination using chloroform : benzene : water (6:4:2) as solvent and I_2 vapours as visualizing agent, indicating it to be a mixture of three compounds A, B and C. These were separated by column chromatography over a silica gel column using $CHCl_3$: MeOH (1:9, 2:8, 3:7) as eluent and purified by preparative TLC, yielding compounds A, B and C.

Study of Compound A

It was crystallized from methanol to yield light brown crystals (1.25 gm) m.p. 262-264°C, m. f. $C_{47}H_{74}O_{16}$, $[M]^+$ m/z 896 (FABMS); Found %: C 62.89; H 8.42; Calcd. % for m.f. $C_{47}H_{74}O_{16}$: C 62.94; H 8.48; IR (KBr): 3430, 2930,1734,1680,1381,1344,1028 cm^{-1}; ^1H NMR (300 MHz, pyridine-d_5) (ppm): δ 3.49 (1H, dd, *J* 10.6, 3.8 Hz, H-3), 5.31 (t, H-12), 1.82 (3H, s, H-29), 1.03 (3H, s, H-30), 0.94, 1.07, 1.12, 1.20, 1.26 (each 3H, s, Me x 5), δ 5.31 (1H, d, *J* 1.8 Hz, H-1'), 4.39 (1H, dd, *J* 1.6, 2.7 Hz, H-2'), 4.08 (1H, dd, *J* 2.3, 8.4 Hz, H-3'), 3.72 (1H, t, J, 8.7 Hz, H-4'), 3.93 1H, m, H-5'), 1.19 (3H, d, *J* 2.4 Hz, H-6', CH_3); δ 5.34 (1H, d, *J* 7.9 Hz, H-1''), 3.86 (1H, dd, *J* 7.5, 8.2 Hz, H-2''), 4.01 (1H, d, *J* 8.4 Hz, H-3''), 4.03 (1H, s, H-4''), 3.68 (2H, d, *J* 9.9, H-5''); δ 5.69 (1H, d, *J* 7.5 Hz, H-1'''), 3.89 (1H, dd, *J* 7.1, 8.2 Hz, H-2'''), 3.62 (1H, dd *J* 8.5, 4.7 Hz, H-3'''), 4.6 (1H, dd, *J* 4.6 , 2.9 Hz, H-4'''), 3.89 (1H, d, *J* 6.1 Hz, H-5'''), 4.31 (2H, dd, *J* 12.7, 4.9 Hz, H-6'''); ^{13}C NMR (75 MHz, Pyridine-d_5) δ 38.37 (C-1), 28.08 (C-2), 79.98 (C-3), 40.12 (C-4), 56.13 (C-5), 19.21 (C-6), 35.02 (C-7), 38.72 (C-8), 48.13 (C-9), 37.92 (C-10), 22.98 (C-11), 126.83 (C-12), 138.11 (C-13), 45.08 (C-14), 28.87 (C-15), 26.44 (C-16), 50.11 (C-17), 133.23 (C-18), 137.86 (C-19), 34.78 (C-20), 31.03 (C-21), 35.67 (C-22), 28.87 (C-23), 16.89 (C-24), 16.04 (C-25), 18.78 (C-26), 21.91 (C-27), 174.27 (C-28), 19.84 (C-29), 18.95 (C-30); δ 105.6 (C-1'), 84.15 (C-2'), 77.18 (C-3'), 70.74 (C-4'), 67.43 (C-5'), 17.92 (C-6', CH_3); δ 102.36 (C-1''), 71.28 (C-2''), 80.4 (C-3''), 72.63 (C-4''), 69.21 (C-5''); δ 104.83 (C-1'''), 74.18 (C-2'''), 74.66 (C-3'''), 70.28 (C-4'''), 76.13 (C-5'''), 60.74 (C-6''').

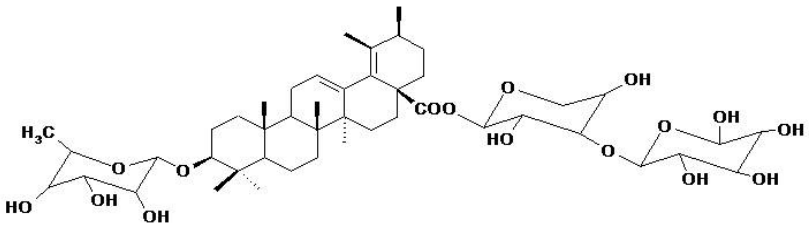

Figure 1 Compound A

Acid hydrolysis of compound A

Compound A (450 mg) was dissolved in ethanol (20 ml) and refluxed with 25 ml of 10% H_2SO_4 on a water bath for 6 hrs. The reaction mixture was concentrated and allowed to cool and the residue was extracted with diethyl ether. The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using $CHCl_3$: MeOH (4:6) to yield compound A-I, identified as 3β hydroxyl-urs-12,18(19)-dien-28-oic acid by comparison of its spectral data with reported literature values. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper

chromatography examination using n-Butanol: Acetic Acid: Water (BAW, 4:1:5) as solvent and aniline hydrogen phthalate as spraying reagent which showed the presence of L-rhamnose (R_f 0.36), D-glucose (R_f 0.17) and D-xylose (R_f 0.27), (Co-PC).

Study of Compound A-I

It had m.p.317-319°C, m.f. $C_{30}H_{46}O_3$, [M^+] m/z 454 (FABMS) Found %: C 79.34; H 10.09; Calcd.% for m.f. $C_{30}H_{46}O_3$: C, 79.30; H, 10.13; IR (KBr): 3422, 2938, 1724, 1676, 1384, 1342 cm^{-1}; ^1H NMR (300 MHz, Pyridine-d_5) : δ 3.46 (1 H, dd, *J* 10.4, 3.5 Hz, H-3), 5.34 (t, H-12), 1.82 (3H, s, H-29), 1.05 (3H, *s*, H-30), 0.92, 1.07, 1.14, 1.21, 1.27 (each 3H, s, Me x 5); ^{13}C NMR (75 MHz, pyridine-d_5): δ 36.56 (C-1), 27.93 (C-2), 79.06 (C-3), 39.81 (C-4), 56.42 (C-5), 18.63 (C-6), 35.14 (C-7), 39.36 (C-8), 48.51 (C-9), 37.62 (C-10), 23.47 (C-11), 126.53 (C-12), 138.16 (C-13), 45.18 (C-14), 28.92 (C-15), 26.54 (C-16), 50.31 (C-17), 133.43 (C-18), 137.86 (C-19), 34.48 (C-20), 31.53 (C-21), 35.60 (C-22), 28.47 (C-23), 16.79 (C-24), 16.34 (C-25), 18.28 (C-26), 21.14 (C-27), 174.67 (C-28), 19.51 (C-29), 18.21 (C-30).

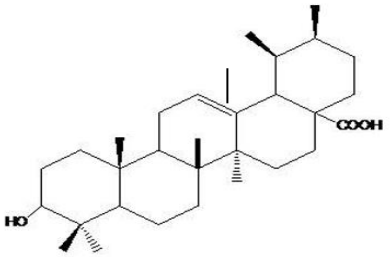

Figure 2 Compound A-I

Permethylation followed by hydrolysis of compound A

Compound A (30 mg) in MeI (10ml) and Ag_2O (20 mg) in DMF (10 ml) was refluxed at room temperature for one day in 100 ml conical flask. The total reaction mixture was filtered and the residue was washed with DMF. The filtrate was concentrated under reduced pressure and treated with $CHCl_3$ (25 ml) and washed with water. After removal of solvent a syrupy mass was found which was hydrolysed with 7% H_2SO_4 (5 ml) to give methylated aglycone and methylated sugars, which were separated by filtration and studied separately. The methylated sugars were identified as 2, 3, 4 tri-O-methyl-L-rhamnose (R_G 1.02), 2, 3, 4, 6 tetra-O-methyl-D-glucose (R_G 0.89) and 2, 4 di-O-methyl-D-xylose (R_G 0.68) (Co-PC and Co-TLC).

Enzymatic hydrolysis of compound A

Compound **A** (30 mg) was dissolved in MeOH (20 ml) and hydrolysed with equal volume of enzyme almond emulsin in a round bottomed flask fitted with air condenser. The reaction mixture was allowed to stand at room temperature for two days and filtered and kept for further studies. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen pthalate as spraying agent which showed the presence of D-xylose (R_f 0.27) and D-glucose (R_f 0.17). Proaglycone on further hydrolysis with enzyme takadiastase yielded L-rhamnose (R_f 0.36) and aglycone identified as 3 hydroxy-urs-12, 18(19)-dien-28-oic acid.

Study of Compound B

It was crystallized from acetone to give light brown needle compound (300 mg) m.p. 285-286°C, m. f. $C_{30}H_{50}O$, $[M]^+$ m/z 426 (FABMS), Found %: C 84.48; H 11.70; Calcd. % for m.f. $C_{30}H_{50}O$: C 84.51; H 11.74; IR (KBr): 3478, 3054, 2995, 2860, 1647, 1472, 1386 cm^{-1}; ^1H NMR (300 MHz, pyridine-d_5) (ppm): δ 5.47 (1 H, dd, *J* 7.7, 3.5 Hz, H-

15), 3.14 (1 H, dd, *J* 11.3, 4.1 Hz, H-3), 1.13 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.82 (6H, s, 2×CH_3), 0.87 (3H, s, CH_3), 0.84 (3H, s, CH_3), 0.73 (3H, s, CH_3), 0.75 (3H, s, CH_3); ^{13}C NMR : (75 MHz, pyridine-d_5) (ppm): δ 37.1 (C-1), 27.4 (C-2), 77.9 (C-3), 38.6 (C-4), 55.9 (C-5), 18.8 (C-6), 34.8 (C-7), 38.1 (C-8), 49.1 (C-9), 37.1 (C-10), 16.7 (C-11), 35.9 (C-12), 36.8 (C-13), 157.2 (C-14), 116.1 (C-15), 35.7 (C-16), 37.9 (C-17), 48.3 (C-18), 40.7 (C-19), 29.0 (C-20), 33.8 (C-21), 32.8 (C-22), 28.4 (C-23), 15.1 (C-24), 14.9 (C-25), 29.2 (C-26), 24.9 (C-27), 29.1 (C-28), 33.5 (C-29), 21.6 (C-30).

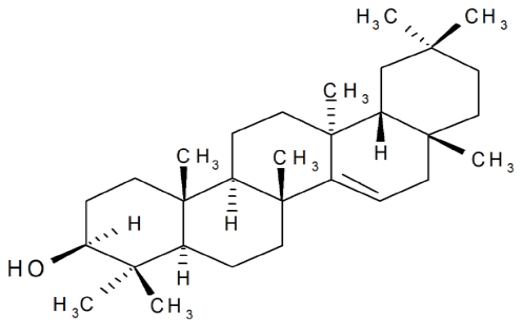

Figure 3 Compound B

Study of Compound C

It was crystallized from methanol to give light yellowish needles (200 mg) m.p. 260-263°C, m. f. $C_{30}H_{50}O$ $[M]^+$ m/z 426 (FABMS), Found %: C 84.48; H 11.70; Calcd. % for m.f. $C_{30}H_{50}O$: C 84.41; H 11.67; IR (KBr): 3428, 2935, 2875, 1730, 1435, 1395, 1172, 1116, 1078 cm^{-1}; ^{1}H NMR (300 MHz, pyridine-d_5) (ppm): δ 0.59 (3H, s, H-24), 0.84 (3H, s, H-25), 0.86 (3H, d, H-23), 0.94 (3H, s, H-30), 0.96 (1H, m, H-22b), 0.99 (3H, s, H-29), 1.03 (3H, s, H-26), 1.08 (3H, s, H-27), 1.15 (3H, s, H-28), 1.19 (1H, m, H-19b), 1.22 (1H, m, H-11b), 1.33 (1H, dd, H-19a), 1.26 (m, H-6b), 1.28 (m, H-12b), 1.29 (m, H-15b), 1.31 (m, H-16b), 1.40 (m, H-7b), 1.38 (m, H-12a), 1.40 (m, H-8), 1.42 (m, H-21b), 1.44 (m, H-11a), 1.46 (m, 22a), 1.47 (m, H-21a), 1.49 (m, H-7a), 1.59 (m, H-10), 1.54 (m, H-15a), 1.56 (m, H-16a), 1.57 (m, H-18), 1.71 (1H, m, H-1b), 1.74 (m, H-6a), 1.87 (1H, m, H-1a), 2.13 (1H, q, H-4), 2.25 (1H, m, H-2b), 2.37 (1H, qd, H-2a); ^{13}C NMR : (75 MHz, pyridine-d_5) (ppm): δ 22.7 (C-1), 41.9 (C-2), 212.8 (C-3), 58.7 (C-4), 42.5 (C-5), 41.4 (C-6), 17.9 (C-7), 52.7 (C-8), 37.9 (C-9), 60.1 (C-10), 36.2 (C-11), 30.1 (C-12), 40.0 (C-13), 38.5 (C-14), 32.6 (C-15), 36.2 (C-16), 29.8 (C-17), 43.2 (C-18), 34.7 (C-19), 27.8 (C-20), 33.1 (C-21), 39.3 (C-22), 6.2 (C-23), 14.9 (C-24), 17.4 (C-25), 19.8 (C-26), 18.9 (C-27), 31.7 (C-28), 32.1 (C-29), 33.8 (C-30).

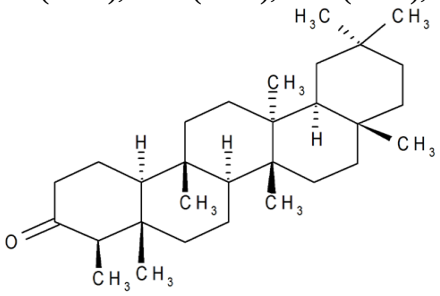

Figure 4 Compound C

Antidepressant activity

The behaviour despair study was performed in order to investigate the ability of herbal drug in elevation of suppressed mood .For this purpose two animal models, Forced Swim Test[9-11] developed by Porsolt et al.[12], (1981) and Tail Suspension Test[13-15] developed by Steru et al.[16], (1985) were employed. The immobility displayed by rodents

when subjected to unavoidable stress such as forced swimming or tail suspension test is thought to reflect a state of despair or lowered mood, which are also thought to reflect depressive disorders in humans[17-18]. Phytochemical screening of *Gloriosa superba* Linn. revealed presence of sterols, tannins, carbohydrates and triterpenoid saponin[19-21]. Triterpenoids present in plants are able to cross blood brain barrier (BBB) due to their lipophilic nature and so it can be assumed that such steroidal compounds might also be responsible to elicit anti-depression and other neuro-pharmacological activities at molecular level in CNS[22-23]. Thus it is likely that triterpenoid present in Gloriosa may be responsible for the observed antidepressant effect.

Results and Discussion

The methanolic extract of the tubers of the plant afforded a new compound A, m.p. 262-264°C m.f. $C_{47}H_{74}O_{16}$, $[M]^+$ m/z 896 (FABMS). It gave yellow color with Liebermann Burchard test confirming it to be triterpenoid saponin[24-26] In the IR spectrum, a peak at 3430 cm^{-1} was assigned for hydroxyl group and 1680 cm^{-1} for double bond. In ^1H NMR spectrum a double doublet at δ 3.49 (1H, dd, *J*10.6, 3.8 Hz) was assigned for H-3. A triplet at δ 5.31 was assigned for H-12. A singlet at δ 1.82 integrating each of the three proton intensity was assigned for H-29. Five signals at δ 0.94, 1.07, 1.12, 1.20 and 1.26 (each 3H, s, Me x 5) confirmed the presence of five CH_3 groups. A singlet at δ 1.03 was assigned for H-30. Three doublets at δ 5.31 (1H, d, *J* 1.8 Hz), 5.34 (1H, d, *J* 7.9 Hz), 5.69 (1H, d, *J* 7.5 Hz) were assigned to H-1', H-1'' and H-1''' of L-rhamnose, D-xylose and D-glucose respectively. The ^{13}C NMR spectrum of compound A exhibited signals for double bond at δ 126.83 and 138.11 between C-12 and C-13 and at δ 133.23 and 137.86 for double bond between C-18 and C-19 respectively. A signal at δ 79.98 suggested the presence of OH group at C-3 and a signal at δ 174.27 confirmed the presence of –COOH group at C-28. Thus the ^{13}C NMR spectrum of compound A exhibited 47 signals, 30 of which could be assigned to the aglycone and 17 signals to the sugar moieties revealing the nature of compound A as bidesmosidic.

Acid hydrolysis of compound A with ethanol and 10% H_2SO_4 gave compound A-I m.p. 317-319°C, m.f. $C_{30}H_{46}O_3$, $[M^+]$ m/z 454 (FABMS). It responded to all the colour reactions of triterpenoids[27-30]. It was identified as 3β-hydroxy-urs-12, 18(19)-dien-28-oic acid by comparison of its spectral data with reported literature values[31-32].

The aqueous hydrolysate obtained after acid hydrolysis of the compound A was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination using *n*-BuOH: AcOH: H_2O (4:1:5) as solvent and aniline hydrogen pthalate as detecting agent yielding L-rhamnose (R_f 0.36), D-xylose (R_f 0.27) and D-glucose (R_f 0.17) (Co-PC)[33].

The sequence of the sugar residue in compound A was confirmed by its partial hydrolysis[34] with Killani mixture[35]. Quantitative estimation[36] of the sugars present in the hydrolysate revealed that the three sugars were present in equimolar proportions (1:1:1) indicating that the saponin consists of one molecule each of, L-rhamnose, D-xylose and D-glucose. Periodate oxidation[37] of compound A confirmed that the three sugars were present in pyranose form.

Permethylation[38] of compound A, followed by acid hydrolysis yielded methylated aglycone identified as 3β-hydroxy-urs-12,18(19)-dien-28-oic acid confirming that glycosidation was involved at 3β hydroxy position and at C-28 position. The methylated sugars were identified as 2, 3, 4, tri-O-methyl-L-rhamnose (R_G 1.02), 2, 3, 4, 6 tetra-O-methyl-D-glucose (R_G 0.89) and 2, 4, di-O-methyl-D-xylose (R_G 0.68) suggesting that C-1''' of glucose was attached with C-3'' of D-xylose and C-1'' of D-xylose was linked to carboxylic group at C-28 position of aglycone. Thus the interlinkage (1→3) was found between D-glucose and D-xylose and also that C_1'-OH of L-rhamnose was directly attached to C_3-OH of aglycone. These linkages were further confirmed by ^{13}C NMR spectral data.

Enzymatic hydrolysis[39] of compound A with enzyme almond emulsin liberated D-glucose (R_f 0.17) followed by D-xylose (R_f 0.27) by (Co-PC) and proaglycone identified as 3β-O-[α-L-rhamnopyranosyl]-urs-12, 18(19)-dien-28-oic acid suggesting the presence of β-linkage between D-glucose and D-xylose as well as between D-xylose and

proaglycone. Proaglycone was hydrolysed with enzyme takadiastase liberating L-rhamnose (R_f 0.36) and sapogenin revealing the presence of α-linkage between L-rhamnose and aglycone.

On the basis of above evidences, the structure of compound A was established as 3β-O-[α-L-rhamnopyranosyl]-urs-12,18(19)-dien-28-oicacid-28-O-[β-D-glucopyranosyl-(1→3)-O-β-D-xylopyranosyl] ester.

Compound B was analyzed for m.f. $C_{30}H_{50}O$, m.p. 285-286°C, [M^+] m/z 426. It was identified as Taraxerol by comparison of its spectral data with reported literature values[40-41].

Compound C was analyzed for m.f. $C_{30}H_{50}O$, m.p. 260-263°C, [M^+] m/z 426. It was identified as Friedelin by comparison of its spectral data with reported literature values[42-43].

Antidepressant activity was evaluated by Behavior despair study in animal model. Forced swim test and Tail suspension test were employed to examine the effect of plant extract on mice and it was concluded from these tests that the plant extract showed antidepressant action on mice used for experiment.

Conclusion

Thus above evidences establish the presence of a new triterpenoid saponin 3β-O-[α-L-rhamnopyranosyl]-urs-12,18(19)-dien-28-oicacid-28-O-[β-D-glucopyranosyl-(1→3)-O-β-D-xylopyranosyl] ester along with two known compounds Taraxerol and Friedelin from the methanolic extract of the tubers of the plant, *Gloriosa superba*. The plant extract when examined for antidepressant activity in animal model by making use of Forced Swim Test and Tail Suspension Test clearly indicated the potential for the use of these herbal drugs as an adjuvant in the treatment of depression. Further research however is required to gain closer insights into the exact mechanism of their action.

Acknowledgements

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