ORIGINAL ARTICLE

Phytopharmaceutical Formulation Development of a Flavonoid Isolated from *Saraca asoca* Bark for Antidepressant Activity

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Abstract

Objective: The objective of the study was to design and evaluate phytopharmaceutical Formulation of flavonoid isolated from *Saraca asoca* bark for antidepressant activity. **Materials and Methods:** A silica gel column (60–120 mesh) chromatography method was used to separate the phytoconstituent from the ethanolic extract of *S. asoca* bark. Direct compression was used to manufacture the mouth-dissolving tablets, which were subsequently assessed for antidepressant activity and other tablet evaluation tests in rats (Institutional Animal Ethics Committee reference number Pharma/FMHS/SGTU/1182), dated 20 September 2021. **Results:** The produced tablets were mechanically sound, as evidenced by their hardness values of 2.95 ± 0.43 kg/cm^2 to 3.85 ± 0.34 kg/cm^2 and friability levels of 0.32–0.54%. Weight variation test results were also within tolerance limits, ranging from 3.01% to 4.54% of the mean weight of 10 pills. The tablets were also found to be consistently within the recommended range of 95–105%, with a drug content uniformity test score of 97.83 ± 1.23% to 100.85 ± 1.22%. Disintegration time, 19 s to 55 s, and dispersion time, 60 s to 115 s, were also fine. The optimized formulation demonstrated antidepressant activity, as evidenced by a significant ($P < 0.05$) reduction in immobility time in both tail suspension test and forced swim test, as well as a significant ($P < 0.05$) reduction in brain tissue glutamate and nitrite levels in mice treated with the formulation compared to the negative control, as did by the standard drug fluoxetine. **Conclusion:** Dispersion time has been used to find out the best the formulation. The prepared best formulation, having a minimum dispersion time of 60 s, that is, F12, was observed to obey with entire physical parameters as well as antidepressant action.

Key words: Antidepressant activity, dispersible tablets, ethanolic extract, *Saraca asoca* bark, phytoconstituent

INTRODUCTION

Depression is a life-intimidating condition that affects millions of persons globally. It can emerge at any stage of life, from childhood to late adulthood, and carries substantial societal costs due to the immense distress and disruption it causes. If left untreated, depression can result in fatal outcomes. The psychopathological state of depression encompasses three primary symptoms: A persistent state of depressed mood, anhedonia (loss of pleasure or interest), and decreased energy levels or fatigue.[1] Furthermore, individuals often experience additional symptoms, including disturbances in sleep patterns and psychomotor functions, experiences of guilt, diminished self-worth, inclination toward self-harm, as well as dysfunctions in autonomic and gastrointestinal processes. The intensity of these symptoms can vary from mild-to-severe, with or without accompanying psychotic

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characteristics, and intricate interconnections with further psychological and physiological ailments.[2]

Despite the availability of various classes of antidepressant drugs, the current treatments for depression have limitations, such as low response rates, late therapeutic effects, and several unwanted effects. Due to various side effects associated with these medicines, it is important to consider herbal remedies as an alternative.[3]

Plant compounds from various categories, including polyphenols (such as coumarins, flavonoids, lignans, and phenolic acids), alkaloids, terpenes, terpenoids, saponins, sapogenins, amines, and carbohydrates, have been discovered to hold antidepressant properties.[4]

These herbal drugs containing flavonoids hold potential for benefiting patients with sub-clinical depression.[5] The isolated flavonoids those could be considered for clinical trials, with the goal of developing therapeutically effective antidepressant treatments.

Saraca asoca is the utmost ancient and sacred tree of India, referred to as an "Ashoka Briksh." Botanically it isreferred to as *S. asoca* (Roxb.) De. Wild or *Saraca indica* and belongs to the family Caesalpiniaceae. Charaka Samhita, supposed to have been unruffled in 1000 BC, defines the Ashoka tree and its therapeutic paybacks.[6]

S. asoca has been nominated as "vulnerable" species by the International Union for Conservation of Nature (IUCN) owing to its continuously falling population as a result of damaging habitat harvesting.[7] Therefore, it is imperative that this species must be conserved and multiplied to get continuous medicinal benefits from it because of its chemical constituents, such as alkaloids and flavonoids.[8] The goal of the present research is to explore the antidepressant activity of flavonoid obtained from the nark of *S. asoca* tree.

In this order, the ethanolic extract of the bark was got, trailed by separation of the possible flavonoid composite by column chromatography using various suitable solvents and combination thereof followed by a suitable formulation development of the same and investigating the formulation for the antidepressant activity.[9]

As far as the formulation development is concerned, fast dissolving formulations were being decided to formulate the isolated flavonoid compound, as literature revealed that flavonoids are poorly soluble in water.[10]

There are various ways, such as particle size reduction, solid dispersion, nanosuspension, super critical fluid process, cryogenic method, inclusion complex-based technique, micellar solubilization, hydrotrophy, and crystal engineering to improve the solubility.[11] Further, Oral drug delivery is thought to be the most affordable, safest, and effective way to administer medication. Easy consumption, self-medication, precise dosage, and patient receipt make it an ideal route for systemic effects.[12] Furthermore, among innumerable pharmaceutical oral preparations, tablets have apparent benefits over other formulations, such as the ease of picking, handling and transportation.[13,14]

Solid formulations have several drawbacks, including a late start of action, and dysphagia (inability to chew or swallow) primarily for elderly and pediatric patients.[15]

To resolve these issues, these solid dosage forms can be suspended or dissolved in saliva. Their disintegration usually occurs in <60 s, and the medication is absorbed by the gastrointestinal system or the oral mucosal tissues in the area.[16] Out of all the flavor masking methods, the coprocessed superdisintegrants method has drawn a lot of interest for use in prescription medicine. This method covers up the undesirable qualities of the individual (such as bitter taste) and allows pharmacological excipients to interact at the subparticle level, improving functionality synergistically. By employing coprocessed superdisintegrants in the formulation of the fast-dissolving tablets, the disintegration time is shortened because the water absorption is increased with a minimum duration of wetting time.[17-19]

The goal of this research is to isolate the flavonoid having antidepressant activity and to create a fast-disintegrating mouth dissolving tablet of the isolated flavonoid using the coprocessed superdisintegrants. This will enable the tablet to dissolve quickly in a minor quantity of water or even in the absence of water, that is, in the saliva only, which could facilitate the rapid onset of action of the newly formulated tablets.

MATERIAL AND METHODS

Materials

Collection and authentication

Bark of *S. asoca* was purchased from Khari Bowli New Delhi and authenticated from NISCAIR. Sample was presented in the Raw material Herbarium and Museum, NISCAIR, New Delhi (Ref No. NIScPR/RHMD/Consult/2022/4060-61).

Chemicals, reagents, and instruments

Ethanol, chloroform, petroleum ether, ethyl acetate, toluene, and silica gel G-60 F254 were purchased from Molychem, Mumbai, India. Polyvinylpyrrolidone, sodium lauryl sulfate, microcrystalline cellulose,and Talc were procured from CDH, New Delhi.

Ultraviolet (UV) spectrophotometer model UV-1601/ SN-A10753984157 (Shimazu Corporation, Kyoto, Japan), USP dissolution apparatus, Electrolab, Model TDT-08L/ SN-0205045, refrigerator (LG company), electronic balance

(FA1104 Electronic Balance), rectangular water bath (VIT company), Fourier transform infrared (FTIR), Agilent technology, Model Cary 630, Microprocessor pH meter, Hanna, Model pH 211, and single station tablet compression machine (Shiva Pharma Engineering India) were used in this study.

Methods

Extraction, isolation, and identification of the flavonoid

Bark was collected and dried at 60°C and minced by using a mechanical grinder trailed by passing the resultant powder through a sieve number 40 and storing in a well closed container until used to prepare the extract.[20]

Preparation of extract

The dried bark powder of *S. asoca* was placed in a Soxhlet extractor and the extraction was then performed using petroleum ether followed by sequential extraction of the residual in chloroform, ethyl acetate and ethanol. The extract gained from each solvent was accurately weighed and yield was recorded. The extracts, thus gained through successive solvent extraction, were qualitatively analyzed to identify various plant constituents.[21,22]

Isolation of flavonoid from the extract

The ethanolic extract, being of the highest yield, was used for further isolation of the flavonoid constituent. The flavonoid constituent was isolated using silica gel (60–120 mesh) column chromatography with the combination of solvent having 7 parts of toluene,5 parts of ethyl acetate, 1 part of formic acid and 0.5 parts of methanol. The fractions were analyzed by thin layer chromatography (using the same solvent system and detected by ferric chloride solution). The fractions having similar retention factor (R_f) values were collected and combined. The fractions, thus combined, were undergone the qualitative tests for the presence of flavonoids, that is, Alkaline test, Shinoda test and Zn-HCl test. The combined fraction was concentrated and kept in refrigerator overnight for crystallization.[9,22]

Identification of the isolated flavonoid constituent

The isolated flavonoid was then undergone identification test using IR spectrophotometry, Proton Nuclear Magnetic Resonance (^{1}H-NMR) as well as Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectroscopy and mass spectrometry for structure determination.

Pre-formulation studies

Solubility studies of isolated flavonoid

Since solubility ultimately influences both drug release from the formulation and drug dissolution, it is an essential property that should be investigated before formulation design. A drug's solubility in water determines how quickly it dissolves and releases its active ingredients. Thus, Solubility plays a role while establishing the final formulation. To evaluate the in-house solubility, the shake flask method was employed. A surplus total of the isolated flavonoid was added into seven closed containers comprising 10 mL water in each container, followed by shaking the containers on an incubator platform shaker for 72 h at 37 ± 0.5°C. The aliquots from each container were then filtered using Whatman filter paper no. 41 and analyzed by UV spectrophotometry in triplicates after suitable dilution at 655nm.[23]

Compatibility studies of isolated flavonoids with excipients

The purpose of the compatibility tests was to make sure that the active component and the excipients would not interact chemically or physically. Physical mixtures of the isolated flavonoid with each excipient were made in a 1:1 ratio to provide even and intimate mixing for the purpose of conducting compatibility experiments. Their IR spectra were then attained and equated with the spectrum of an isolated flavonoid, that is, the reference spectrum for classifying peaks. Physical mixtures were then transferred to separate vials; after adding 5% moisture to each, the vials were closed with rubber stopper which were then hermatically sealed. All samples were kept for 1 week-3 weeks at 55°C and checked for discoloration, caking, liquefaction and odor during these 1–3 weeks frequently. IR spectra of these samples were also attained after 3 weeks and were compared with that of pure active pharmaceutical ingredient (API) for identifying peaks of the isolated flavonoid.[24]

Preparation of coprocessed superdisintegrant

The solvent evaporation approach was used to produce the coprocessed superdisintegrant. The first step involved meticulous blending a 3:1 combination of sodium starch glycolate and crospovidone and adding it to 65 mL of isopropyl alcohol. The mixture was agitated on a magnetic stirrer at 65–70°C, till evaporation of practically entire isopropyl alcohol. After that, a 60-mesh sieve was used to granulate the wet coherent material. Next, freshly produced granules were dried for 20 min at 60°C.After being sieved through a 60-mesh screen, the dried granules were then packed in an airtight container until they were needed again.[17-19]

Formulation of mouth dispersing tablets

The compositions of various batches of mouth-dispersible tablets of the isolated flavonoid have been presented in Table 1. All the batches were compressed into tablets by the direct compression method. All the components, except talc, were correctly weighed, passed across a sieve number 60, and then mixed in a polybag for 15 min (till uniform mixing).

Table 1: Composition of various batches of mouth dissolving tablets of petunidin-3-O-β-glucopyranoside

Ingredients	F10	F11	F12	F13	F14	F15	F16	F17	F18
Drug (isolated flavonoid)	46	46	46	46	46	46	46	46	46
Cross carmellose sodium	10	20	-	-	-	-	10	20	-
Cross povidone	-	-	-	10	20	-	10	20	-
Polyvinylpyrollidone	40	40	40	40	40	40	40	40	40
Coprocessed superdisintegrant	-	-	20	-	-	15	-	-	10
Mannitol	100	100	100	100	100	100	100	100	100
Sodium lauryl sulphate	20	20	20	20	20	20	20	20	20
Talc	8	8	8	8	8	8	8	8	8
Microcrystalline cellulose	176	166	166	176	166	171	166	146	176
Total weight (mg)	400	400	400	400	400	400	400	400	400

Talc was then added to this powder mixture as lubricant. At last, the final mixture was blended for 5 min followed by evaluation for various powder characteristics, such as bulk density, tapped density, Carr's Index, Hausner's Ratio, and Angle of Repose to get the flow properties of the powder blend necessary for compressing the powder into the tablet. The powder blends from each batch were then directly compressed into tablets of average weight 400 mg using a single station hand operated compression machine.[25,26]

Evaluation of powder blend

Bulk density

After cautiously filling a graduated cylinder of 100 mL capacity with a powder sample weighed 20 g, it was allowed to fall twice at 2 s pauses using a bulk density device, and the bulk density was determined by means of the below mentioned equation[27]-

$$\text{Bulk Density} = \frac{\text{Mass of Powder}}{\text{Bulk Volume}}$$

Tapped density

Using bulk density equipment, 20 g of powder was tapped into a measuring cylinder until the volume remained constant. The tapped density was then calculated as follows[27]-

$$\text{Tapped Density} = \frac{\text{Mass of Powder}}{\text{Tapped Volume of Powder}}$$

Carr's index (Compressibility index)

The Compressibility Index, or Carr's index, is widely used in pharmaceutics to gauge how compressible a powder is. A free-flowing powder would have a low Carr's index because the gap between its bulk density and its tapped density would be too small. A larger Carr's index would result from a discrepancy in the claimed bulk density and tapped density, which would be greater in a weakly flowing powder with stronger interactions between the particles. Carr's index was determined as below[27]-

$$\text{Carr's Index} = \frac{\text{Tapped Density-Bulk Density}}{\text{Tapped Density}} \times 100$$

Hausner's ratio

Hausner's Ratio may be used to predict a powder sample's propensity to compress and is thought to highlight the importance of interparticle interactions. A larger gap between the bulk and tapped densities is seen in poorer flowing materials because they have more inter-particle interactions. Hausner's ratio can be calculated by[27]-

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

Angle of repose

The greatest angle involving the edge of a heap of powder sample and its horizontal plane is known as the angle of repose (ϕ), which symbolizes the frictional forces in a loose powder sample. If we add more powder to the pile, it glides over its edges until the friction between particles is in balance with the force of gravity.

To get the "Angle of Repose," the powder was allowed to flow across a vertical funnel followed by measuring the height (h) and radius (r) of the cone shaped heap formed and applying the following formula[27]-

$$\text{Angle of response } (\varphi) = \text{Tan}^{-1}\left[\frac{h}{r}\right]$$

Compression of powder blend into tablets

Powder combinations were directly compressed into tablets by means of single punch manual tablet punching machine.[28,29]

Evaluation of compressed tablets

Hardness

To confirm that the tablets will withstand abrasion and/or breakage on handling during storage, transportation and usage, Test for hardness (kg/cm^2) was performed. A tablet was placed between two anvils of a Monsanto Hardness Tester, the force was applied and the crushing strength that led to breakage of tablet was noted.[30]

Friability

To check that the tablets were mechanically sound to bear the shocks during handling and storage, the friability test was done using the Roche Friabilator. The test was done on tablets sample similar to 10 times the average weight of tablets, that is, 6.5 g, as the average weight of the tablets was 0.400 g, that is, <0.650 g. Exactly weighed tablets sample was permitted to drop100 times from 6 inches height in a Friability Chamber rotating at 25 rpm followed by removal of the whole sample from the friability chamber, de-dusting and accurate weighing. Friability was then calculated using following formula[30]-

$$\%\text{Friability} = \text{Weight Loss of the Tablet Sample}$$

Initial Weight of the Tablet Sample

$$\%\text{Friability} = \frac{\text{Weight Loss of the Tablet Sample}}{\text{Initial Weight of the Tablet Sample}} \times 100$$

Weight variation

To check the drug content uniformity, the test for weight variation was executed. 20 tablets were arbitrarily selected and weighed separately. The average weight of these 20 tablets was then calculated, followed by the calculation of the difference in weight of each tablet from that of the average. Not more than two of the discrete weights vary from the average weight by more than 5% and none vary by >10%.[30]

Drug content uniformity test

Drug content in each of 10 dosage units of the sample was distinctly determined as per IP 2010. The tablet was crushed and dissolved in 100 mL methanol with resilient shaking for 10 min trailed by centrifuge. The supernatant was then collected. The process was repeated twice with the residue with same quantity of fresh methanol. All supernatants were mixed and assessed spectrophotometrically at 655 nm for drug content after suitable dilution.[30]

Disintegration test

The disintegration test was conducted to verify that the tablet broke apart into smaller pieces to facilitate the dissolving and, hence, drug release from the tablet, as the solvent infiltration into the tablet is tough because of compression followed by consolidation. Six tablets were randomly selected from each batch to measure the disintegration time in water at 37 ± 0.5°C and put one tablet each in the six tubes of the apparatus. The apparatus was then operated in the water-filled beaker till all the six tablets become disintegrated so as to get the disintegration time.[30]

Dispersion time

Dispersion time was restrained to ensure that the prepared tablets follow the criterion of super disintegration in the mouth only. The test was performed *in vitro* by submerging the tablets sample in 10 mL of water. A smooth dispersion should be formed that passes through a sieve having 710 µm aperture.[30]

Water absorption ratio

It is a key indicator to know the ability of disintegrating pharmaceuticals to swell even in small amounts of water, which subsequently claims the drug dissolution. A twice-folded piece of tissue paper was kept in each of the 6 petri-dish (internal diameter 9 cm) containing 9 mL of water. A tablet was positioned on the paper, and the amount of time needed for it to completely wet was recorded. Following complete wetting, the tablets were weighed. Ultimately, the water absorption ratio (WR) of each tablet was determined using the formula below, followed by calculation of the mean and standard deviation too.[31]

$$WR = \frac{W_a - W_b}{W_b} \times 100$$

Where, Wa and Wb are the respective weights (mg) of the tablet after and before absorption of water.

Antidepressant activity studies of the prepared formulation of the isolated flavonoid

Every formulation, that is, F10-F18passed every assessment test, and the optimal formulation was taken into consideration depending on dispersion time. The preparation with the shortest dispersion time has thus been viewed as the best one. After getting approval from the Institutional Animal Ethics Committee, dated 20 September 2021, under reference number Pharma/FMHS/SGTU/1182, a few behavioral studies, that is, locomotion activity, tail suspension test (TST), forced swim test (FST), and whole brain neurochemical assays, that is, brain nitrite and glutamate assay[32] were done on 3-4 month-old male Swiss albino mice of 20-30 g weight (purchased from AIIMS, New Delhi) at SGT University, Gurugram, Haryana, India, to determine the antidepressant activity of the optimal formulation.

Mice were housed in the central animal house at 21–23°C with a 12-h each light and dark cycle beginning at 7:00 h and 19:00 h, respectively. Feed and water were freely available to them.

For TST, the doses as existing in Table 2 were administered orally in mice and the latter were suspended by tail for 6 min and the immobility period was noted (n = 6).[33]

For FST, the mice were given oral doses as presented in Table 2 and kept in a cylindrical container (Height 30 cm and Diameter 20 cm) filled to the height of 15 cm with water at 25°C and immobility period was measured for 6 min (n = 6).[34]

A 10 %w/v brain homogenate in phosphate buffer pH 7 ± 0.1 at 4°C was deproteinated with 10% trichloroacetic acid followed by centrifuging for 20 min at 12000 rpm in a cooling centrifuge at 4°C.[33,34]

The brain glutamate test involved adding 300 µL of brain homogenate to 100 µL of phosphate buffer, pH of 7.0 ± 0.1, followed by pH adjustment to 9.0 ± 0.1 and left aside in an ice bath for 10 min. Glutamate was then measured spectrophotometrically at 340 nm.[33-35] The findings are shown in Table 2.

The brain nitrite study involved adding 100 µL of brain homogenate to 100 µL of the Griess Reagent, letting it stand aside for 10 min at room temperature, and then measuring the amount of nitrite at 546 nm using spectrophotometry.[36,37] The findings are shown in Table 2.

Where appropriate, the test results were statistically assessed by One-Way Analysis of Variance.

RESULTS AND DISCUSSION

Identification of the isolated flavonoid

The isolated flavonoid was then undergone identification test using IR spectroscopy [Figure 1], ^{1}H-NMR [Figure 2] as well as ^{13}C-NMR [Figure 3] spectroscopy and mass spectrometry [Figure 4] for structure determination.

FTIR, wave number, cm^{-1}; 3411.14, 3105.03, and 3063.48 (-OH, CH_3/CH_2), 1625.31, 1587.95, and 1501.08, (C=O, C=C), 1389.36 and1258.75 (diketones and C-O-C), 1052.33 and 997.95 (aromatic and aliphatic proton scissoring vibrations) [Figure 1].

1H-NMR (CD3OD, 400MHz: δ 10.051, 9.476, 9.001 and 7.117 (4H, 2s, d, 1st, 3rd, 13th and 14th hydroxy group), 6.481, (1H, d, Ar, CH-6), 6.032 (3H, m, Ar, CH-2, 12, 16), 5.689 (1H, d, Ar, CH-23), 5.094 (3H, t, for three hydroxy group (3OH) of 26th, 27th and 28th position of carbons of glucose ring), 4.483 (2H, m, for 30th and 28th position of hydroxy group), 4.169 (3H, s, 15th position of methoxy group; -OCH_3), 3.989, 3.649 and 3.291 (5H, 25th, 26th, 27th and 30th position of carbon group in glucose ring) [Figure 2].

13C-NMR (CD3OD, 400MHz: δ 168.53, 163.22, 158.76 and 158.02 (4C, at 1st, 3rd, 5th, 9th position of carbon), 149.90, 145.99 and 135.09 (3C, at 15th,13th and 14th carbon of dihydroxy methoxy phenyl ring), 145.08 and 139.99 (2C, 8th and 7th carbon position of chromenylium ring), 123.72, 109.97 and 109.85 (3C at 11th,12th and 16th carbon of dihydroxy methoxy phenyl ring), 114.03, 103.08 and 97.35 (3C, 4th, 2nd, and 6th position of carbon in chromenylium ring), 108.91, 83.38, 76.98, 76.35, 74.11 and 64.35 (6C, 23rd, 25th, 27th, 28th, and 26th carbon position of glucose ring), 59.62 (1C of methoxy group) [Figure 3].

MS (ESI) m/z: 480.20 (M+H) [Figure 4]

As interpreted by IR spectrum, ^{1}H-NMR and ^{13}C-NMR spectra, and mass spectrum of the isolated flavonoid in the above mentioned paragraphs, the structure as per Figure 5 has been confirmed that can be chemically named as petunidin-3-O-β-glucopyranoside (PGLU).

Solubility is required before formulation design as it eventually disturbs the dissolution of the drug as well as its release pattern from the formulation. It is also studied to define the solubility category of a drug. A solubility of 0.023 g/mL indicates that the isolated flavonoid PGLU can be categorized as sparingly soluble in water, that is, necessitate 50 parts (More than 30 parts and <100 parts) of water for one part of PGLU to get entirely dissolved. Hence, the solubility of PGLU required to be upgraded during formulation design.[38] Hence, mouth-dissolving dispersible tablets have been planned and gaged as rationalized in the introduction section.

Table 2: Parameters for antidepressant activity

S. No.	Group (Dose)	TST (Immobility Period, Sec)^	FST (Immobility Period, Sec)^	Locomotion Activity, Sec^	Glutamate Level (µM)^	Nitrite Level (µM)^
1.	Vehicle (10*)	144.50±21.72	23.67±6.28	33.83±6.91	0.67±0.19	11.28±2.14
2.	Negative control (ND)	203.33±44.86	63.17±14.71	49.67±10.65	1.34±0.16	17.16±2.30
3.	Standard drug (Fluoxetine) 10**	142.83±66.60	24.83±2.79	40.00±3.26	0.81±0.28	11.12±1.48
4.	F12 (PGLU) 100**	145.16±11.47	22.33±6.25	33.83±5.71	0.81±0.30	6.79±0.94

^Mean±Standard deviation (n=6), *mL/kg body weight, ND: No Dose, **mg/kg body weight, PGLU: Petunidin-3-O-β-glucopyranoside, TST: Tail suspension test, FST: Forced swim test

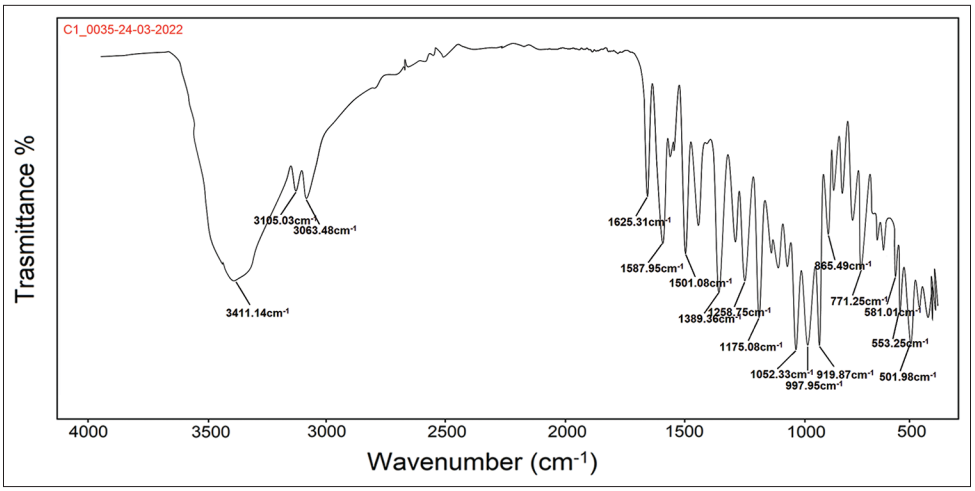

Figure 1: Infrared spectrum of the isolated flavonoid form ethanolic extract of the *Saraca asoca* bark

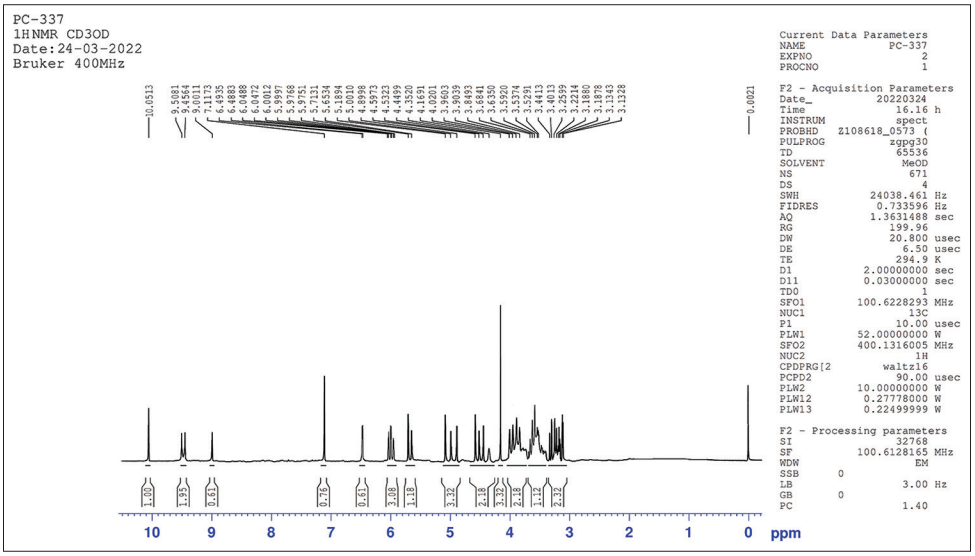

Figure 2: Proton nuclear magnetic resonance spectrum of the isolated flavonoid form ethanolic extract of the *Saraca asoca* bark

The formulation design has been presented in Table 1. Before crafting of the formulation batches, drug-excipients incompatibility studies have been executed by IR spectroscopy and the results have been written in Tables 3 and 4.

There were no indications of physical interactions, such as caking, liquefaction, discoloration, or the development of odor [Table 3]. In addition, the existence of certain peaks of the PGLU in the infrared spectra of the physical blends at zero time and subsequently the 3rd week of storing confirms that the drug and the excipients are chemically compatible [Table 4].[39] The micromeretic properties of all formulations' powder combinations were examined to validate the flow characteristics of the powder blends that were best suited for tablets. Table 5 displays the micromeretic characteristics of the produced blend of powder. It was noted that all of the flow property metrics, including Carr's Index, Hausner's Ratio, and Angle of Repose, fell between the permitted ranges of 15.64% and 23.27%, 1.18 and 1.30, and 22.33° ± 1.04° to 30.23° ± 1.92°, respectively.[30]

As evidenced by the numerical values of Carr's Index or Compressibility Index, Hausner's Ratio, and Angle of Repose, the tablets can be formed from the powder mixtures of all the batches by direct compression deprived of granulation.

The prepared directly compressed tablets were estimated for the official as well as unofficial post-compression parameters, such as hardness, friability, weight variation, drug content, disintegration time, and dispersion time. Results are presented in Table 6.[40]

The hardness, as determined by a Monsanto Hardness Tester, ranged from 2.99 ± 0.35 kg/cm^2 to 3.85 ± 0.34 kg/cm^2 [Table 6]. This means that the produced tablets will be able to tolerate handling, storage, and transit without breaking or abrasion. A 0.32–0.49% weight loss [Table 6] after friability test, that is, forever <0.5% for the batches F11, F12, and F14-F18, designates that the tablets from these lots were mechanically sound and can survive the mechanical shockwaves throughout handling and storage. Formulations

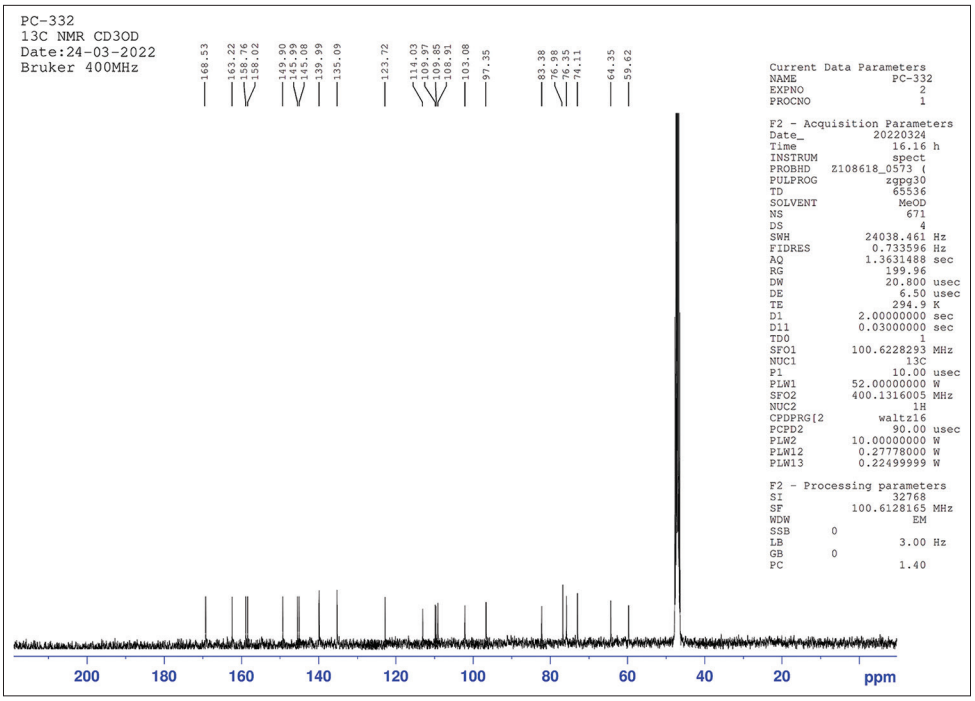

Figure 3: Carbon-13 nuclear magnetic resonance spectrum of the isolated flavonoid form ethanolic extract of the *Saraca asoca* bark

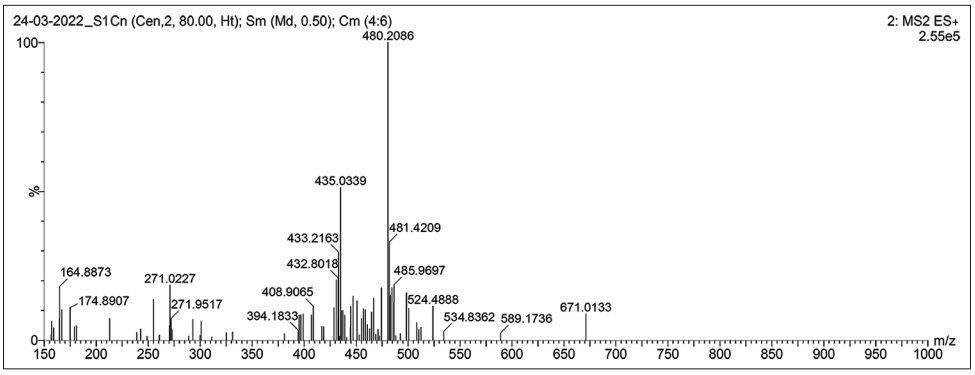

Figure 4: Mass spectrum of the isolated flavonoid form ethanolic extract of the *Saraca asoca* bark

Table 3: Physical observations of samples stored at 55°C with 5% moisture over three weeks

Parameters	Caking	Liquefaction	Discolouration	Odour Formation
Result	-	-	-	-

F10 and F13 fails the test for friability as indicated by more than 0.5% loss in weight during the test.

The tablets from all the batches passed the test for weight variation [Table 6], as shown by 3.01–4.54% variance in the individual tablet weight from average weight of 10 Tablets (data were given only for the formulation of the tablets found to have maximum difference in weight) which is forever less than acceptance limits of 5% for the tablets having average weight >324 mg.

It became essential to do the drug content analysis in addition to the weight variation analysis since the uniformity of mixing test was not conducted. It was determined that the drug content for all formulations, that is, F10 to F18, was between 97.83% ± 1.23% and 100.85% ± 1.22% [Table 6]. This means that all the ready batches qualified the drug content analysis test because the percent content uniformity of the drug was consistently within the specified limits of 95–105%.

The disintegration and dispersion times of 19–55 s and 60–115 s, correspondingly, are specified in Table 6 to guarantee that compression and the resulting consolidation would not significantly impact drug dissolution. The prepared tablets have been found to pass the criteria of dispersible

Table 4: Solid-state compatibility of PGLU with excipients: IR spectroscopy data

Wave Number recorded for physical mixture of PGLU and excipients (cm^{-1})		Absorption frequency band (cm^{-1})	Characteristic functional group/vibration
0 time	3rd week		
3411.14	3415.15	3200–3600	Alcoholic OH (Aromatic) Functional Group
1625	1625.64	1636	C=C in 2-H Chromene Ring
3063.48	3050.61	2800–3100	CH (Aromatic) Stretching
1175.08 and 1052.33	1200.13 and 1050.05	1220 and 1025	Ar-O-C (Substituted C_5O Aliphatic Ring) bending
865.49 and 771.25	855.14 and 764.11	900-690	Aromatic C-H bending in a substituted Benzene ring
1570	1571	1566	C=C Skeletal in Aromatic Ring
997.95	995.23	992	Transition from β-D-glucose to methyl-β-D-glucopyranoside

PGLU: Petunidin-3-O-β-glucopyranoside, IR: Infrared

Table 5: Flow and compressibility parameters of the prepared powder blend: buLk density, tapped density, Carr's index, Hausner ratio, and angle of repose

Batch	Bulk density (g/mL)*	Tapped density (g/mL)*	Carr's index (%)**	Hausner's ratio**	Angle of repose (°)*
F10	0.38±0.04	0.49±0.03	22.86	1.29	25.32±1.54
F11	0.45±0.06	0.55±0.08	17.12	1.20	25.43±1.22
F12	0.34±0.03	0.45±0.05	23.27	1.30	22.33±1.04
F13	0.41±0.05	0.49±0.09	16.43	1.19	30.23±1.92
F14	0.44±0.02	0.54±0.05	18.99	1.23	29.65±1.54
F15	0.41±0.04	0.51±0.06	19.58	1.24	28.28±1.24
F16	0.43±0.01	0.52±0.05	17.31	1.20	26.54±1.35
F17	0.46±0.07	0.55±0.05	15.64	1.18	29.45±1.25
F18	0.43±0.05	0.52±0.01	17.91	1.21	26.33±1.23

*Mean±Standard deviation for 3 Measurements. **Calculated from mean values of bulk density and tapped density

Table 6: Post-compression parameters for prepared mouth dissolving tablets

Batch	Hardness (kg/cm^2)*	Friability (%)	Weight variation (%)**	Drug content (%)*	Disintegration time (Seconds)	Dispersion time (Seconds)	Water absorption (%)
F10	3.55±0.21	0.51	4.13	100.03±1.21	55	115	59.15
F11	3.11±0.14	0.49	3.14	97.83±1.23	49	100	63.27
F12	2.95±0.43	0.33	3.01	98.02±1.34	19	60	95.25
F13	3.85±0.34	0.54	4.54	99.16±2.01	53	115	58.75
F14	3.14±0.25	0.47	3.22	98.43±1.87	45	101	63.11
F15	3.11±0.19	0.44	4.33	98.62±1.53	26	65	91.15
F16	3.65±0.45	0.32	4.46	100.85±1.22	45	101	65.19
F17	2.99±0.35	0.48	3.99	98.03±2.01	39	85	70
F18	3.75±0.34	0.49	4.23	98.12±1.91	36	75	85

*Mean±Standard deviation for *n*=3 measurements. **%Difference of weight of the tablet from the average weight of 10 Tablets (Data provided only for the tablet found to have the maximum difference)

tablets as indicated by complete passage of the produced smooth dispersion through a 710 μm sieve.

Out of all the formulations, that is, F10 to F18, F12 has been taken as best because of minimum period of dispersion (dispersion time), that is, 60 s [Table 6]. Thus, the F12 formulation [Table 1] was considered for further antidepressant activity.

Various batches' water absorption ratios were found to range between 58.75% and 95.25% [Table 6]. The water absorption ratio and disintegration time showed an inverse relationship

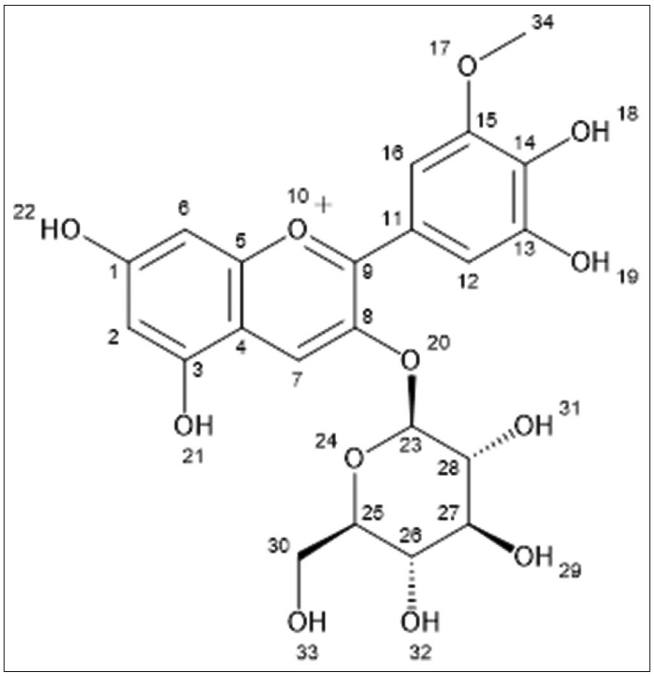

Figure 5: Structure of the isolated flavonoid form ethanolic extract of the *Saraca asoca* bark as confirmed by the interpretation of acquired infrared spectrum, proton nuclear magnetic resonance and carbon-13 nuclear magnetic resonance spectra, and mass spectrum

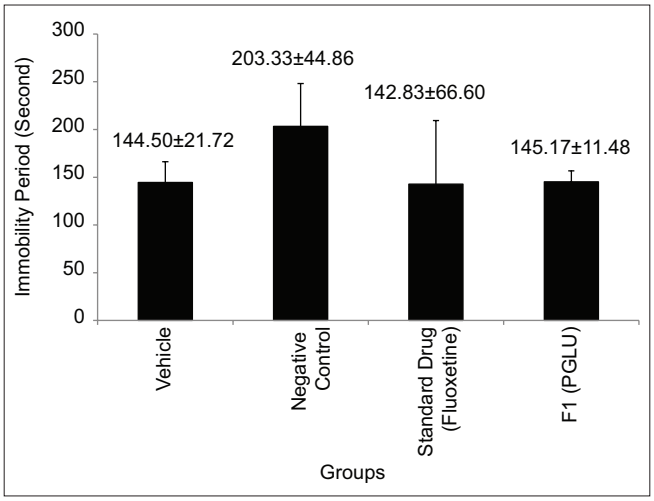

Figure 6: Effect of various treatments on the immobility period of mice in the tail suspension test using an actophotometer (mean ± standard deviation, $n = 6$ in each group); $P < 0.05$, indicates significant difference from the group treated with the vehicle

for every batch. In other words, the batch with a shorter disintegration time had more water absorption, and vice versa. Further, water absorption ratio is a key factor to recognize the strength of disintegrants to swell even in little amount of water, which later captivates the drug dissolution.[41]

Behavioral studies (such as the locomotion activity, TST, and FST) and whole brain neurochemical assays (such

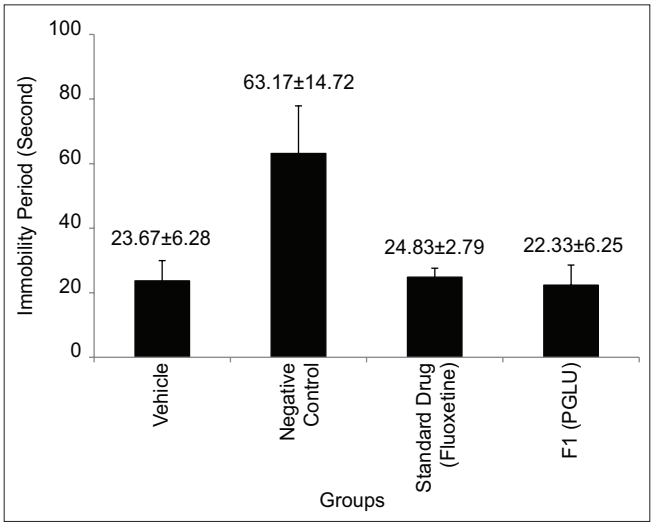

Figure 7: Effect of various treatments on the immobility period of mice in the forced swim test using an actophotometer (mean ± standard deviation, $n = 6$ in each group); $P < 0.05$, indicates significant difference from the group treated with the vehicle

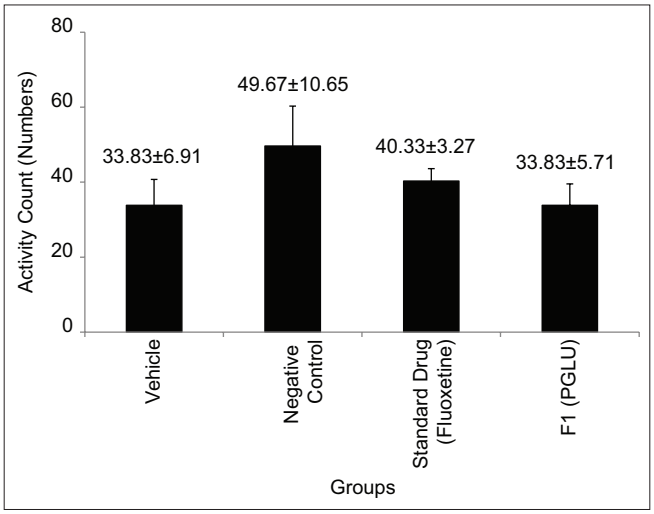

Figure 8: Effect of various treatments on the immobility period of mice in locomotionactivity test using actophotometer (mean ± standard deviation, $n = 6$ in each group); $P < 0.05$, indicates significant difference from the group treated with the vehicle

as the brain nitrite and glutamate assays) were used to investigate the antidepressant activity. The findings are shown in Table 2 and Figures 6-10. The TST and the FST were created as behavioral indicators to forecast the impact of antidepressants. These techniques are straightforward and yield trustworthy outcomes.

According to the results [Table 2], the test medication PGLU considerably ($P < 0.05$) reduced immobility time in the TST and FST as compared to negative control, which exhibits antidepressant-like behavior in mice ($n = 6$), just as much as standard control fluoxetine did. Similarly, mice treated with PGLU ($n = 6$) showed antidepressant-like behavior as their brain tissue glutamate ($P < 0.05$) and nitrite ($P < 0.05$) levels

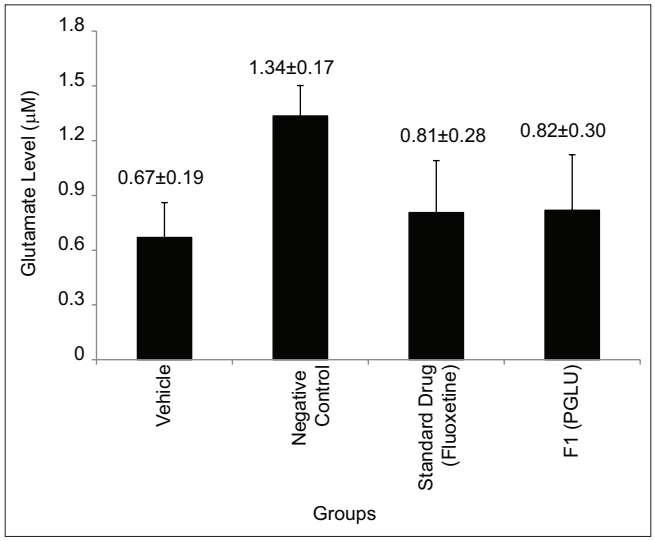

Figure 9: Effect of various treatments on the glutamate level of mice (mean ± standard deviation, n = 6 in each group); $P < 0.05$, indicates significant difference from the group treated with the vehicle

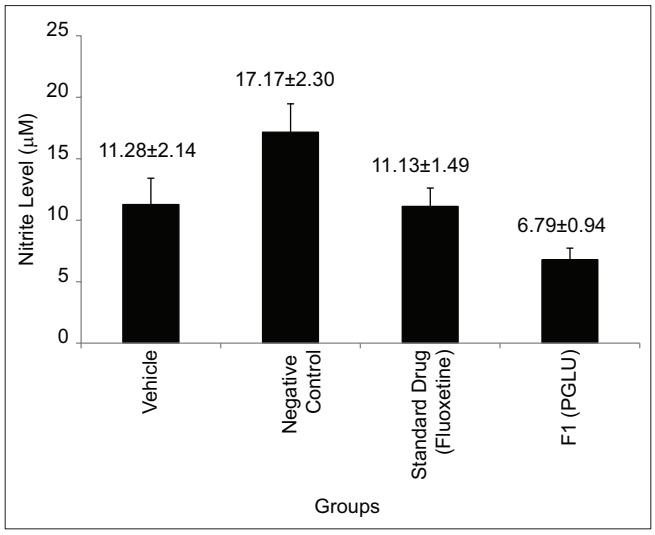

Figure 10: Effect of various treatments on the nitrite level of mice (mean ± standard deviation, n = 6 in each group); $P < 0.05$, indicates significant difference from the group treated with the vehicle

were considerably lower than those of the negative control (n = 6) and the conventional medication fluoxetine (n = 6). On the other hand, no discernible variation in locomotor activities was found.

CONCLUSION

Dispersion time has been used to find out the best formulation. The prepared best formulation, having a minimum dispersion time of 60 s, that is, F12, was found to adhere to all physical parameters as well as antidepressant activity.

AUTHOR'S CONTRIBUTIONS

Each author has an equal contribution.

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