Universal Research and Academic Journal

E-ISSN: 2584-2838

(Peer Reviewed, Refereed and Multidisciplinary Journal)

Universal Research and Academic Journal

Volume: 3, Issue: 3, (March-2024), pp: 01-11

Received: 05th March, 2024 *Accepted:* 13th March, 2024 ©URAJ: 2024/03/001/011/001

Invivo Adaptogenic Activity Assessment of Sambucus nigra and Streblus asper: Standardization of Polyherbal Syrup, Investigation of Quercetin and Kempherol

Sachin Neekhra[1*], Alok Kumar Shukla[2], Shravan Kumar Paswan[3], Shikhar Verma[4], Himani Awasthi[5]

[1*]Babu Sunder Singh College of Pharmacy, Nigoha, Lucknow, (sachinpharma13@gmail.com)

[2]Babu Sunder Singh College of Pharmacy, Nigoha, Lucknow

[3]Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow

[4]School of Pharmaceutical Sciences, Maharishi University of Information Technology Lucknow

[5]Hygia Institute of Pharmaceutical Education & Research, Lucknow

Abstract: People's concern in traditional medicine has increased recently; however, customary herbal medicines need to be converted into a modern system of medicine to increase patient recognition. Objective - The present work includes the fabrication and characterization of polyherbal syrup with hydroalcoholic extractof Sambucus nigra and Streblus asper leaves. Methods - Physicochemical analysis of selected crude drug was done. Various physicochemical evaluations of polyherbal syrup such as pH, density, specific gravity, viscosity, refractive index and accelerated stability testing were determined. The qualitative determination of a flavonoidal biomarker (Quercetin and Kaempferol) was performed via HPTLC. Antistress activity was assessed using foot-shock induced stress model. Twenty albino rats (5 mice in each group) were grouped into normal control, stress control, polyherbal syrup (200 mg/kg) and diazepam (1 mg/kg). Polyherbal syrup treatment was given orally for 2 weeks with prior use of foot shock induced stress.

Results - The study given above shows result data which are obtained from physio-chemical analysis of selected crude herbs for syrup preparation showed physicochemical characters, accelerated stability testing of polyherbal syrup was validated & standardised as per Ayurvedic pharmacopoeia limit. A significant variation was found in number of mountings, duration of immobility and active avoidance response in foot shock induced stress as compared to stress control group. The research data approved that polyherbal syrup showed significant anti-stress potential which attributes to the explored biomarkers (Quercetin and Kaempferol) and the stability studies of standardized formulation justify its shelf life.

© **Corresponding Author:** Mr. Sachin Neekhra

Keywords- Anti-stress activity, Kaempferol, Polyherbal syrup, Quercetin, Sambucus nigra, Streblus asper

1. **INTRODUCTION**: The acceptance of traditional, complementary and alternative medicine has grown globally. Because of the increasing use of plant-based medicines and the prompt expansion of the worldwide market for these goods, the well-being and quality of herbal plant materials and finished herbal medicines have become a major issue for the public health establishment[1].There is significant diversity in the quality control of such materials and products, and this variety has an influence not only on community health, since toxins in herbal medicines may represent avoidable dangers for users, but also on intercontinental trade[2]. To reduce the risk of adverse events caused by unsafe and low-quality herbal medicines, the International Conference of Drug Regulatory Authorities (ICDRA) and the National Centres participating in the WHO Drug Monitoring Programme asked WHO to develop and continuously revise technical guiding principles on the quality, safety, and efficacy of herbal medicines.Standardization is the process of evaluating the physicochemical properties of crude drugs, the efficacy, safety, and consistency of finished products, the credentials of safety and risk based on experience, the provision of product information to consumers, and the endorsement of products[3](Chandel et al., 2011). Traditional medicinal systems have the potential to be a source of novel drugs. These experiments were carried out in order to design, develop, and evaluate the phytochemical properties of poly herbal syrup.Kaempferol and quercetin are particular markers from Sambucus nigra and Streblus asper that are used to treat a range of illnesses, including neurological problems, epileptic illnesses, Parkinson's disease, and Alzheimer's disease[4](Khan et al., 2020). Stress has been associated in the etiopathogenesis of a wide range of illnesses, including mental illnesses like depression and anxiety, immune-suppression, endocrine disorders like diabetes, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension, and ulcerative colitis[5-7](Jaggi et al., 2011; Palomba et al., 2018; Salleh, 2008).Polyherbal syrup contains hydroalcoholic extract of *Sambucus nigra* and *Streblus asper* L. All of the constituents in Syrup have been extensively researched in Ayurvedic literature and scientific research journals for the management of physical and mental stress, general debility, and rehabilitation. They are also mentioned for having anti-oxidant and anti-stress potentials. However, there was no proof that their combination was safe or effective. The current study attempted to examine Polyherbal Syrup's in-vitro anti-oxidant property and in-vivo anti-stress activity.

© Corresponding Author: Mr. Sachin Neekhra

2. MATERIALS AND METHODS

2.1 Plant materials

Leaves of selected plant *Sambucus nigra* were collected from Forest Research of India, Dehradun and *Streblus asper* leaves were collected from LakheempurKheeri, U. P, India.The herbariums were prepared in triplicate and submitted and authenticated from CSIR- National Botanical Research Institute (NBRI), Lucknow (NBRI/CIF/526/2016), and Forest Research of India (272/2016/syst. Bot/ Rev/4-5).

2.2 Chemicals

HPTLC studies were performed using, Merck 20.0 cm × 10.0 cm silica gel 60 F_{254} (0.20 mm) pre-coated plates. All solvents, reagents and compounds used such as,Quercetin (Sigma-Aldrich), Sodium chloride (SD Fine Chemicals Ltd), Sodium hydroxide (Thermo Fisher scientific India Pvt. Ltd.), Hydrochloric acid (Merck Darmstadt, Germany), Ethanol (Qualikems Fine Chemicals, New Delhi), Toluene (Merck Darmstadt, Germany), Ethyl acetate (Merck Darmstadt, Germany), Formic acid (Merck Darmstadt, Germany), Tri-ethanolamine (CDH), Sodium benzoate (Merck Darmstadt, Germany), Citric acid (SD Fine Chemicals Ltd.) and glycerin (SD Fine Chemicals Ltd.), Propyl paraben (Himedia labs Pvt. Ltd.) were purchased by the institute for the research work of analytical grade.

2.3 Preparation of Extracts

Leaves of *Sambucus nigra* and *Streblus asper* were properly washed, shade dried and powdered to 40 mesh and subjected to solvent extraction separately. The extraction was carried out for both plant materials separately for 16 h with the hydro-alcohol (70% v/v; 300 mL) using soxhlet apparatus. The obtained hydroalcoholic extracts were concentrated under vaccum at 40ºC using rota vapour. Dried powdered extracts were stored under refrigeration at 4ºC till further use. The dried extracts were dissolved in 98% methanol to obtain a stock solution of 10 mg/mL, which is used for the application of bands on HPTLC plates.

2.4 HPTLC finger printing

2.4.1 Instrumentation and chromatographic conditions

The stock solutions with a concentration of 10 mg/mL were also applied on a pre coated HPTLC silica gel 60 F254 on aluminum sheets to a band width of 10mm using CAMAG Linomat 5 – applicator. Stock solution plate was developed in Toluene: Ethyl acetate: Formic acid in the ratio of 6.5:2.5:1 respectively. Slit dimensions: 6.00×0.45 mm. Scanning speed: 10 mm s–1 and source of radiation: deuterium lamp. The plate was scanned at 340 nm and Rf, colour spots and densitometric scan were recorded[8, 9](Ariyanathan et al., 2010; Kabra et al., 2019).

2.4.2 Calibration curve of quercetin and kaempferol

© Corresponding Author: Mr. Sachin Neekhra

Stock solutions of quercetin and kaempferol (1 mg/mL) were prepared in HPLC grade methanol. Different volumes of stock solutions were spotted on the TLC plate to obtain concentrations of 100–600 ng per band of quercetin and kaempferol, respectively.

2.5 Preparation of Polyherbal syrup

Distilled water (10 mL) was taken in conical flask and then heated and boiled. When boiling was started, add 13.34 gm sucrose slowly with constant stirring until it became homogenize syrup. Sodium benzoate, Citric acid and glycerin were dissolved separately in 2 mL distilled water. It was mixed and heated until all ingredients dissolved properly. Took 6 mL of distilled water in separate conical flask and add to it 200 mg of hydroalcoholic extract of *Sambucus nigra* and 200 mg of hydroalcoholic extract of *Streblus asper*. Add the filtered solution to homogenized syrup prepared previously, stirred and mix for 20 min. The syrup was cooled and volume was made upto 20 mL with distilled water[10, 11](Khanum et al., 2016; Sheikh et al., 2014).

2.5.1 Physicochemical quality control of the polyherbal syrup

2.5.1.1 Physical examination- Macroscopic characteristics like color (brown colour), odor, taste and appearance of the syrup were analysed.

2.5.1.2 pH:pH of the formulated syrup was measured at room temperature.

2.5.1.3 Density:Density of the syrup was measured in triplicate at room temperature by using a 10 mL pycnometer.

2.5.1.4 Viscosity measurement:The viscosity was calculated by placing 600 mL of the syrup in a Brookfield viscometer; Spindle No.2 with 30 gear speed at room temperature. The test was done three times[10, 12, 13](Khanum et al., 2016; Ordoñez et al., 2006; Yu et al., 2002).

2.5.1.5 Short term thermal stability

Three bottles of syrup were placed in refrigerator (4° C) and another three samples in incubator at 40° C. Seven days later, the samples were replaced. After the fourteen-day's cycle, the samples were periodically evaluated for changes like sedimentation, taste, odor and color.

2.5.1.6 Accelerated stability tests

Six bottles of syrup were placed in a preheated oven (at 40 ° C.). After 3 and 6 months, they were examined based on the above-mentioned measures[14](Shah et al., 2021).

2.6 Administration of the polyherbal syrup and diazepam

The diazepam was suspended in 2% gum acacia, administrated intraperitoneally (i.p.) before 30 min of induction of stress. Polyherbal syrup of dose level 200 mg/kg was administrated 1hr before the induction of stress.

2.7 Pharmacological Screening

2.7.1 Animals Used in Study

The Institutional Animal Ethical Committee gave its clearance to the animal studies (Approve Ref No(SRGI/ COP/A/29/2016, CPCSEA Reg No. 1624/PO/a/CPCSEA).Adult, pathogen-free Wistar rats of male sex with a body weight of 160±20g were used. They were housed in plastic cages with clean bedding and had unrestricted access to food and water. The animals were acclimatised for one week before the study, and the animal room was kept at a constant temperature (25±2 °C) and humidity (50% ± 5%), with a 12-hour light/dark cycle.

2.7.2 Foot-shock induced stress

Stress protocol described by Armando was used. (Armando I., 1993) During 14 consecutive days, Footshock stress through a grid floor in a shock chamber was applied to rats for 1 h daily. The duration of each shock (2 mA) and the interval between the shocks was randomly programmed between 3 and 5 sec and 10 and 110 sec, respectively. After the last shock procedure on day 14, other test procedure was performed. (Nade et al., 2009)

2.7.2.1 Cognitive Dysfunction

Active Avoidance Test was used to investigate the consequence of stress on retention of a learned task as memory:

2.7.2.2 Behavioural despair test

Rat was forced to swim in vessels (45×40×30 cm) separately with a water level of 20 cm, so the foot of the rat did not touch the vessel's floor or scramble out of it.The rat had 10 minutes' swimming time. The whole immovability duration was recorded in the next 5 minutes as characterized by a complete swimming stop with a head floating above the water level. This moment of immobility was designed to show 'conductual despair' after heated attempts to flee(Hill and Gorzalka, 2005).

2.7.2.3 Active Avoidance Test

Rats are trained before they are exposed to stress for an active avoidance task. The rats were placed on the grid floor throughout the training and allowed to get used to it for 5 minutes. Then the rats were subjected to an electric shock (1 mA, 50 Hz) supplied by a buzzer stimulus 15 sec (conditioned stimuli) (unconditioned stimulus). On day 1 of the test protocol, rats had at least 10 trials at an interval of 60 min, until the 100% avoidance response requirement of jumping to the non-electrified wooden pole was achieved. On day 14 the trial process was again conducted to examine how the active avoidance learning is retained(Singh et al., 2012).

2.7.2.4 Sexual Behavior

A male rat was placed in a cage in a dimly-lit room for 10 min with 2 oestrinized (sequentially treated with oestradiol valerate 5 µg/rat, followed 48 hr later by hydroxyprogesterone 1.5 mg/rat, sc) female rats, the parameters observed as total number of mounts was counted(Bhattacharya et al., 2000).

© Corresponding Author: Mr. Sachin Neekhra

2.8 Statistical analysis

All the values are expressed as mean ± SEM. Statistical differences between means were determined by one way ANOVA followed by Dunnett's post hoc test. $P < 0.05$ was considered as significant. The statistical analysis was done using Instat ® software (Graph pad Inc., Santabarba, CA).

3. RESULTS

Table 1: Quantification of Quercetin and Kaempferol in HPTLC

Quantification of quercetin and Kaempferol in hydro-alcoholic extracts of *Sambucus nigra* and *Streblus asper*

S. No.	Plant Sample	Quercetin (%)	Kaempferol (%)
1	*Sambucus nigra*	0.13	0.24
2	*Streblus asper*	0.08	0.27

Table 2: Summary of validation parameters

S. No.	Parameters	Quercetin	Kaempferol
1	R_F	0.55	0.65
2	Linearity range(ng)	100-600	100-600
3	Regression via area	y=12.822*x+524.747	y=12.267*x-884.577
4	R	0.999	0.998
5	Slope	12.822	12.267
6	Intercept	524.747	-884.577
7	LOD (ng)	25	28
8	LOQ (ng)	75.75	84.84
9	Scanning (nm)	340	340

Symbol is for multiplication in the equation: Y = mx + c

Table 3: Physicochemical analysis of selected crude drug

The physicochemical analysis of the raw materials which include total ash, acid insoluble, foreign matter, loss on drying, water soluble extractive and alcohol soluble extractive were provided in table 3.

S. No.	Crude drug	Total Ash value (%)	Acid insoluble ash (%)	Foreign matter (%)	Loss on drying (%)	Alcohol soluble extractive (%)	Water soluble extractive (%)
1	*Sambucus nigra*	6.23	0.57 ± 0.04 (NMT 2)	0.5 ± 0.42 (NMT 9)	5.89% (NMT 10%)	32.69± 0. 85	56.58± 2.19
2	*Streblus asper*	8.113	0.06± 0.00 (NMT 0.2)	0.2 ± 0.01 (NMT 2)	10.09± 0.38	39.71± 0. 81	44.57± 1.19

Table 4: Physicochemical characteristics of Polyherbal syrup

The formulated Polyherbal syrup has reddish brown in colour with characteristic odour and taste. The pH of polyherbal syrup was slightly acidic. Viscosity and density of polyherbal syrup were found uniform and given in **Table 4**.

S. No.	Physicochemical parameter	Tests
1	Colour	Reddish brown
2	Taste	Sweet
3	Appearance	Semi transparent
4	Viscosity (mPa s)	19.8
5	Density (g/mL)	1.2051
6	pH	4.8

Table 5: Evaluation of physicochemical parameters and results of accelerated stability testing of polyherbal syrup

© **Corresponding Author:** Mr. Sachin Neekhra

S. No.	Specification	Start	3rd Months	6th Months
1	Appearance	Light brown semi-transparent liquid	Conforming	Conforming
2	Taste	appropriate	Conforming	Conforming
3	Odor	Characteristic	Conforming	Conforming
4	pH	4.8	4.7	4.7
5	Sedimentation (%)	0.019	0.016	0.016
6	Specific gravity (g/mL)	1.2051	1.1093	1.1093
7	Viscosity (mPa)	20.2	20.5	20.5
8	Conclusions on stability		Stable	Stable

Fig. 1A HPTLC image at 340 nm, 1B and 1C. HPTLC Chromatogram of *Sambucus nigra* (*S. nigra*) of *Streblus asper* (*S. asper*)

Study revealed, at 340 nm got 2 spots, densitometric scan at 340 nm revealed 2 peaks corresponding to 2 different compounds in the syrup, compounds with Rf - 0.55, 0.65 (quercetin and kaempferol).

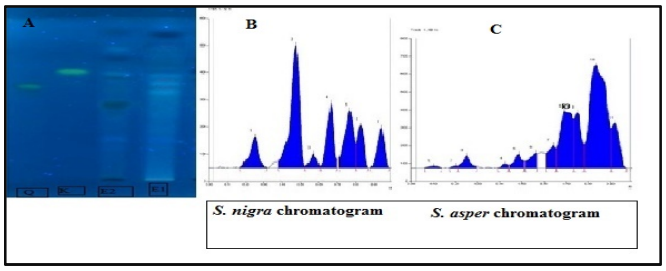

Fig. 1BHPTLC chromatogram of standard of quercetin and Kaempferol

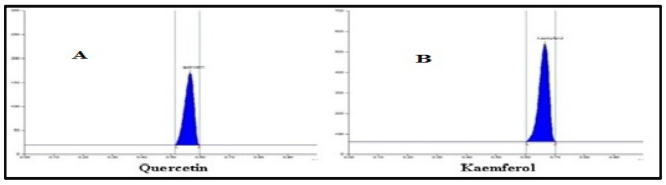

Table 6: Effect of polyherbal syrup on neuro and sexual behaviour changes and swim stress immobility in rats

Footshock stress significantly increased the duration of immobility as well as decreased the sexual behaviour (number of mounting) and the retention of acquired active learning. Polyherbal syrup (200 p.o.) and diazepam (1mg/kg i.p.) significantly reversed (**P < 0.1, ***P < 0.05) stress induced neuro and sexual behaviour changes as indicated by decreased the duration of immobility, increased number of mountings in **table 6** and retention of acquired active learning in **table 7**.

S. No.	Treatment group	Number of mounting (N)	Duration of immobility	
			Day 1 (Sec)	Day 14 (Sec)
1	Normal Control (10 ml/kg)	10 ± 0.58	122.8 ± 1.15	125.8 ± 0.66
2	Stress Control (10 ml/kg)	4.2 ± 0.73###	170.6 ± 0.92###	239.6 ± 1.8###
3	Polyherbal syrup (200 mg/kg)	9 ± 0.7***	125.6±7.4**	165 ± 6.3***
4	Standard drug diazepam (1 mg/kg)	9.6 ± 0.5***	123.5 ± 4.2***	154.790 ± 1.8***

Value are expressed as mean ± SEM (n = 5), one way ANOVA followed by Dunnett's test; ###P< 0.1, ***P < 0.05, when compared with the stress group

Table 7: Effect of Polyherbal syrup on memory deficit in active avoidance response

S. No.	Treatment group	Active avoidance response on Day 14 (%)
1	Normal Control (10 ml/kg)	80
2	Stress Control (10 ml/kg)	20[a]
3	Polyherbal syrup (200 mg/kg)	70[b]
4	Standard drug diazepam (1 mg/kg)	73.37[b]

Values are expressed as percentage way (*n* = 5), one way ANOVA followed by Dunnett's test;

[a]$P<0.001$ when compared to normal control [b]$P<0.5$, when compared with the stress group.

4. DISCUSSION

Herbal remedies have been utilised for the prevention and treatment of many ailments since ancient times. In recent years their use has increased dramatically[15, 16](J.B., 2000; Shivakumar et al., 2016).Traditional remedies can be employed as novel therapeutics in research, but they must be modified to meet contemporary drug pharmacopoeia requirements. The lack of quality control profiles for plant formulations motivates scientists to learn more about them. The importance of formulation quality control in ensuring the product's safety and efficacy is critical.

Syrup formulation was prepared in accordance with Indian traditional medicine in the current investigation. Experiment syrup included active substances and necessitated the addition of excipients such as preservatives, viscosity-increasing agents, and sweetening agents. So, to get the confirmed viscosity for the poly-herbal syrup, there was addition of glycerin. Further there was investigation of properties of experimental formulation it manifested that glycerin has enhanced the taste & visual appearance & elevated the viscosity of the syrup. So, glycerin it used as a sweetener, agent which enhanced the viscosity, co-solvent, stabilizer& as well as transparency enhancer all this was used in the syrup formulation. There was also addition of sodium benzoate & citric acid in the form of anti-microbial preservative & anti-oxidant agent. When the evaluation of the quality of syrup control was done it has shown that the optimum visual property was seen without any evidence of crystallization. Also, there as not any indication of physical changes was seen in the short-term thermal stability tests & centrifugation process. As the results came under continuous stability test it has been shown in Table 3. The parameters evaluated were odour, taste, pH, sedimentation % etc give the physio-chemical properties of syrup after 3 & 6 months. Then after 3 & 6 months there was decrease in sedimentation percentage & density were found to be decreased. Then on the basis of the results of accelerated stability test, there was no statically changes ($p \geq 0.05$) were found about the measured parameters in the 6 months this further confirmed about the stability of the product. After this, it is concluded that the polyherbal syrup has showed the stability after the six months at 40 regarding its physical characters & it was further accepted for an oral product.

When the body is unable to cope with the stress situation, various biological and behavioural events of external as well as internal stress occurs, which may perturb homeostasis and also leads to various clinical diseases. So, to understand these factors, stress research seems to be essential in laboratory animals[17](Huether, 1996).The

method used in this study employs mild electroshock Stress, unpredictable in nature, administered over a period of two weeks. Chronic causes endogenous depression, according to a vast amount of clinical and experimental research. Uncontrollable stress is used in a number of animal models of depression, and the biochemical correlates of such testing are similar to those observed in chronic stress.Chronic stress is known to affect other endocrine responses as well, which can induce sexual debility in males[18](Adriaan Bouwknecht et al., 2007). Foot shock characterizes the unpredictable and inescapable nature of stress[19](Bhattacharya et al., 2002). Chronic exposure to foot shock disrupts body's own adaptation process and results stress mediated perturbations including cognitive dysfunction, and behavioural depression[20](Nade et al., 2009). Polyherbal syrup and diazepam reversed chronic stress induced inhibition of male sexual behaviour. Stress has been shown to interfere with cognitive activities, causing memory engrams to be delayed rather than learning to be acquired.

The mechanisms involved in the memory-attenuating effect of stress remains conjectural but a similar neurochemical basis operating in the induction of stress-induced depression, may be responsible[16, 21](Muruganandam et al., 2002; Shivakumar et al., 2016). Polyherbal syrup and diazepam attenuated the stress induced deficit of retention of learned tasks.Thus, the dose 200 mg/kg of polyherbal syrup has markedly affected behaviour perturbations in which exhibited nearly effect to that of standard diazepam drug. The present investigation shows that the polyherbal syrup has significant adaptogenic activity as shown by its alleviating effects on several chronic stress induced behaviour perturbations, comparable to that induced by the well-established standard adaptogenic drug diazepam.

CONCLUSION

Herbal remedies have been utilised for the prevention and treatment of many ailments since ancient times. In recent years their use has increased dramatically. Traditional remedies can be employed as novel therapeutics in research, but they must be modified to meet contemporary drug pharmacopoeia requirements. The lack of quality control profiles for plant formulations motivates scientists to learn more about them. The importance of formulation quality control in ensuring the product's safety and efficacy is critical. The Polyherbal syrup consisting of thehydroalcoholic extractof *Sambucus nigra* and *Streblus asper* leaves with antistress activity in male rat was prepared and these were evaluated and standardized. The extracts were quantified with HPLC for the lead compound by using biomarkers Quercetin & Kaempferol. The accelerated stability study (40°C ± 2° C / 75% RH ± 5%) for 3 and 6 months indicates that the formulation is stable under these conditions. This

© **Corresponding Author:** Mr. Sachin Neekhra

Polyherbal syrup needs to be further evaluated for its activity in clinical trials.

ACKNOWLEDGEMENT

The author gratefully acknowledges work supported by Dr. Shikhar Verma, Principal, Maharishi School of Pharmaceutical Science, Maharishi University of Information Technology, Lucknow.

CONFLICT OF INTEREST

There is no conflict of interest into the manuscript.

REFERENCE

1. Parmar, S., et al., *DETERMINATION OF QUERCETIN BY HPTLC METHOD PRESENT IN ZYMODYNE SYRUP-A POLY HERBAL FORMULATION.* International Journal of Pharmaceutical Sciences and Research, 2011. **2**(10): p. 2724.
2. Yadav, N. and V. Dixit, *Recent approaches in herbal drug standardization.* Int J Integr Biol, 2008. **2**(3): p. 195-203.
3. Chandel, H.S., A. Pathak, and M. Tailang, *Standardization of some herbal antidiabetic drugs in polyherbal formulation.* Pharmacognosy research, 2011. **3**(1): p. 49.
4. Khan, A., et al., *Antioxidant and anti-inflammatory effects of citrus flavonoid hesperetin: Special focus on neurological disorders.* Antioxidants, 2020. **9**(7): p. 609.
5. Jaggi, A.S., et al., *A review on animal models for screening potential anti-stress agents.* Neurological Sciences, 2011. **32**: p. 993-1005.
6. Palomba, S., et al., *Lifestyle and fertility: the influence of stress and quality of life on female fertility.* Reproductive Biology and Endocrinology, 2018. **16**: p. 1-11.
7. Salleh, M.R., *Life event, stress and illness.* The Malaysian journal of medical sciences: MJMS, 2008. **15**(4): p. 9.
8. Ariyanathan, S., A. Saraswathy, and G. Rajamanickam, *Quality control standards for the roots of three Plumbago species.* Indian journal of pharmaceutical sciences, 2010. **72**(1): p. 86.
9. Kabra, A., et al., *Pharmacognostic characterization of Myrica esculenta leaves.* Journal of Ayurveda and integrative medicine, 2019. **10**(1): p. 18-24.
10. Khanum, K., et al., *Clinical studies of linkus syrup for efficacy and safety for the treatment of cough, respiratory infection in children.* RADS Journal of Pharmacy and Pharmaceutical Sciences, 2017. **5**(2): p. 36-43.
11. Sheikh, Z.A., et al., *Design, development and phytochemical evaluation of a poly herbal formulation linkus syrup.* Chinese Medicine, 2014. **2014**.
12. Ordonez, A., J. Gomez, and M. Vattuone, *Antioxidant activities of Sechium edule (Jacq.) Swartz extracts.* Food chemistry, 2006. **97**(3): p. 452-458.
13. Yu, L., et al., *Free radical scavenging properties of wheat extracts.* Journal of agricultural and food chemistry, 2002. **50**(6): p. 1619-1624.

14. Shah, P., et al., *Formulation development of linagliptin solid lipid nanoparticles for oral bioavailability enhancement: Role of P-gp inhibition.* Drug delivery and translational research, 2021. **11**: p. 1166-1185.
15. Calixto, J., *Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents).* Brazilian Journal of medical and Biological research, 2000. **33**: p. 179-189.
16. Shivakumar, A., et al., *Pharmacognostic evaluation of triphala herbs and establishment of chemical stability of triphala caplets.* Int J Pharm Sci Res, 2016. **7**(1): p. 244-51.
17. Hüther, G., *The central adaptation syndrome: psychosocial stress as a trigger for adaptive modifications of brain structure and brain function.* Progress in neurobiology, 1996. **48**(6): p. 569-612.
18. Bouwknecht, J.A., B. Olivier, and R.E. Paylor, *The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse.* Neuroscience & Biobehavioral Reviews, 2007. **31**(1): p. 41-59.
19. Bhattacharya, A., et al., *Effect of poly herbal formulation, EuMil, on neurochemical perturbations induced by chronic stress.* 2002.
20. Nade, V.S., et al., *Adaptogenic effect of Morus alba on chronic footshock-induced stress in rats.* Indian journal of pharmacology, 2009. **41**(6): p. 246.
21. Muruganandam, A., V. Kumar, and S. Bhattacharya, *Effect of poly herbal formulation, EuMil, on chronic stress-induced homeostatic perturbations in rats.* 2002.