Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2019, Volume 10, Issue 12, Page 1230-1237

http://www.jmaterenvironsci.com

Poly Phenolics-Rich Extracts of *Lagerstroemia indica* and *Salvia splendens* Attenuate Behavioural Alterations and Oxidative Damage of Reserpine–Induced Depression in Rats

Salma A. El-Sawi[1], Rana M. Merghany[1], Amany. A. Sleem[2], Hanan F. Aly[3], Mohamed E. Ibrahim[4]

[1]*Dept. of Pharmacognosy, National Research Centre Cairo-Egypt,*
[2]*Dept. of Pharmacology, National Research Centre Cairo-Egypt,*
[3]*Department of Therapeutical Chemistry, National Research Centre Cairo-Egypt,*
[4]*Dept. of Research of Medicinal and Aromatic Plants, National Research Centre, 33 El Buhouth St., (P.O.12622), Giza, Egypt*

Received 22 Sept 2019,
Revised 25 Oct 2019,
Accepted 27 Oct 2019

Keywords
- ✓ Antidepressant;
- ✓ Antioxidant;
- ✓ *Lagerstroemia indica* ;
- ✓ *Salvia splendens;*
- ✓ Depression*;*
- ✓ Behavior;
- ✓ Rat.

selsawi7@yahoo.com
Phone: +201224215109

Abstract

Depression is a serious and common disease that negatively affects how you can think, feel, and act. Depression can cause feelings like loss of interest and sadness. In this study, we examined for the first time the efficacy of different extracts of aerial parts of *Lagerstroemia indica* (F. Lythraceae) and *Salvia splendens* (F. Lamiaceae) as antioxidants and anti depressants *in vivo*. The study includes phytochemical and physical analysis of the two plants. *In vitro* antioxidant activity using DPPH method was performed on all extracts of the two plants to determine the most active extracts to be injected *in vivo* in reserpine-induced depression rat models and evaluate their efficacy as anti depressants using Porsalt's forced swimming test. Extracts prepared with ethyl acetate and ethanol (polar solvents) for the two plants proved to be the most active extracts as antioxidants at a concentration of 300 µg/ml with percent of inhibition 72.26 and 83.20 %, respectively, for *Lagerstroemia indica* and 42.30 and 94.16 %, respectively, for *Salvia splendens*. Ethyl acetate and ethanol extracts of both plants were injected *in vivo* to evaluate their effect in controlling behavioral depression. Both extracts of the two plants (100 mg/Kg) showed significant reduction ($P < 0.05$) from the normal control in immobility time for the rats to swim. Preliminary phytochemical screening performed, showed that poly phenolic compounds may contribute in these activities. *L. indica* and *S. splendens* proved to be as natural alternatives in managing behavioral depression.

1. Introduction

Depression disorder may lead to chronic or recurrent mood disorders as well as disturbances in behavioral and cognitive. Although, different antidepressant synthetic treatments are available now to manage its complications, alternative medicines or crude extracts produced from natural sources such as plants, microbes and animals, also invaded the field of controlling and treating neurodegenerative disorders due to their availability and safety [1].There is a direct pharmacological relation between oxidative stress and depression-like effects in rat models where antioxidant treatments banned these behaviors, signifying the causal role of oxidative stress in this complication [2]. Oxidation reactions can involve the production of free radicals, which can form dangerous chain reactions and produce different physiological and behavioral changes. Antioxidants can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by being oxidized themselves. Natural antioxidants especially anthocyanins, phenolics and flavanoids are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used to control many other complications.

It is believed that the human body finds plant-derived medicines easier to accept due to the fact that they exist in nature and are not synthetic. About 25% of prescription medicines in the USA are believed to have an active ingredient from a natural source. In developing countries, it's estimated that about 80% of their populations rely on traditional medicines made from plants and herbs [1].
The Lamiaceae family comprises about 200 genera and 3000 species. One of the largest genera of the family, genus *Salvia*, is represented by over 900 species that were used worldwide in folk medicines and for culinary purposes, ranging from aches to epilepsy, and mainly to treat colds bronchitis, tuberculosis, hemorrhage, and menstrual disorders [3]. On the other hand, Lythraceae is a family of flowering plants, including 32 genera with about 620 species. The genus *Lagerstroemia* is a genus of around 50 species of deciduous and evergreen trees, began to attract the attention of scientists worldwide due their biologically active compounds [4]. In the present study, *in vitro* DPPH free radical scavenging activity and *in vivo* anti depressant like effects of the different extracts of both *S. splendens and L. indica* were examined, also preliminary phytochemical screening was performed to determine the major class of active compounds that may contribute to the biological activities.

2. Materials and Methods

2.1.Plant material:

Samples of *L. indica* and *S. splendens* aerial parts were obtained from El-Orman botanical garden and were kindly authenticated by Mrs. Tereize Labib, Agricultural Engineer, El-Orman botanical garden, Giza, Egypt. Voucher specimen of *L. indica* was deposited at Faculty of Pharmacy, Ain Shams University (PHRM-1508), where for *S. splendens*, a voucher specimen (Reg. No. 5.1.2016) was deposited at Faculty of Pharmacy, Cairo University. The air-dried aerial parts of the two plants were powdered and known weights of the powder were exhaustively extracted by refluxing with 80% methanol to yield the total extract. Another portion of the powder was defatted using petroleum ether (40-60°C) followed by chloroform then ethyl acetate and finally ethanol in a Soxhlet apparatus to yield four successive fractions. All solvents were evaporated to dryness under reduced pressure at 40°C and weighed.

2.2. Phytochemical screening:

- Test for carbohydrates and/or glycosides using Molisch's reagent [5].
- Test for sterols and/or triterpenes using Lieberman-Burchard reagent [6, 7].
- Test for tannins using Ferric chloride reagent [8].
- Test for flavanoids, free and combined using Aluminum chloride reagent [9].
- Tests for anthraquinones, free and combined [10, 11].
- Test for alkaloids and/or nitrogenous compounds using Mayer's reagent [12].
- Tests for saponins [13].
- Test for coumarins [14].

2.3. Chemicals:

1, 1-diphenyl-2-picrylhydrazil (DPPH) (Sigma® Chemical Company (EUA)). Vitamin C (Cid Co., Egypt). Fluoxetine hydrochloride (Prozac 20 mg, dispersibine tablets, lilly, Alcobendas, Spain).

2.4. *In vitro* DPPH scavenging activity:

The antioxidant of serial concentrations of different plant extracts (100:400 µg/ml) was estimated using DPPH method. The reduction in $DPPH^{\cdot}$ optical density is estimated relative to control as: % Inhibition $= [(A_{control} - A_{sample}) / A_{control}] \times 100$. Vitamin C was used as a reference drug applying the same conditions [15].

2.5. *In vivo* antidepressant activity:

2.5.1 Animals:

Male wistar albino rats 120 - 150 g were used. The animals were attained from the animal house of National Research Centre (Egypt). Anesthetic techniques and usage of animals obeyed the ethical guidelines of Medical Ethical Committee of National Research Centre in Egypt with approval number 14147. Control groups were fed with standard diet (El- Kahira Co. for Oil and Soap). Doses of the drugs were calculated according to the results of LD_{50} of investigated extracts [16, 17], and been administered orally by oral tubes.

2.5.2. Experimental design:

Depression is a neurological behavior which was induced in rats (groups 2,3,4,5,6 and 7) by reserpine injection (0.25 mg/kg s.c.), every 48 hours for 20 days. The most active antioxidant extracts of the two plants and fluxoetine

(standard) were administered orally daily during 20 days before the behavioral test. Rats were divided into 7 groups; Group 1: Control Normal. Group 2: Control Depressed. Group 3: *L. indica* ethyl acetate extract (EAE) (100 mg/Kg). Group 4: *L.indica* ethanol extract (EE) (100 mg/Kg). Group 5*: S. splendens* EAE (100 mg/Kg). Group 6*: S. splendens* EE (100 mg/Kg). Group 7: Fluoxetine drug (20 mg/kg) [18].

2.5.3. Porsalt's forced swimming test:

Each animal was placed individually in a water tank of diameter 40 cm, height 70 cm. The tank was filled with water at height of 30 cm at temp. 22-23 ºC. Then the animal was forced to swim for 6 min and the duration of immobility was measured. An animal was considered as immobile when it stopped struggling and moved only to remain floating in water, keeping head above water the floating time. This is used as the measure of despair and recorded after treatment [19].

2.6. General Statistical analysis method:

Data are presented as mean ± S.E.

From six animals in each group; statistical significance was evaluated using ANOVA test followed by posthoc Duncan's multiple range test. A probability value of less than 0.05 was considered statistically significant.

3. Results

3.1.Preparation of the different extracts of the plants:

The yields of the different extracts of *L. indica* and *S. splendens* are presented in Table 1.

Table 1. Yield of different extracts of *L. indica* and *S. splendens*

Extract	Wt. of the used plant powder (gm)		Yield (gm)	
	L. indica	***S. splendens***	***L. indica***	***S. splendens***
TE	**50**	**150**	**11.50**	**30.00**
PEE	**360**	**550**	**11.50**	**9.50**
CE	**360**	**550**	**5.50**	**8.50**
EAE	**360**	**550**	**7.00**	**3.00**
EE	**360**	**550**	**42.00**	**30.00**

Where TE: total extract, PEE: petroleum ether extract, CE: chloform extract, EAE: ethyl acetate extract and EE: ethanol extract.

3.2. In vitro DPPH scavenging activity:

For *L. indica*, Fig. 1 showed significant decrease in inhibition percent of its total extract at several concentrations as compared to vitamin C. The highest inhibition percent is detected at 100 µg/mL reached to 80.29 %, while gradual decrease in inhibition percent at 200, 300 and 400 µg/mL (53.28, 28.46 and 4.37 %, respectively) was observed.

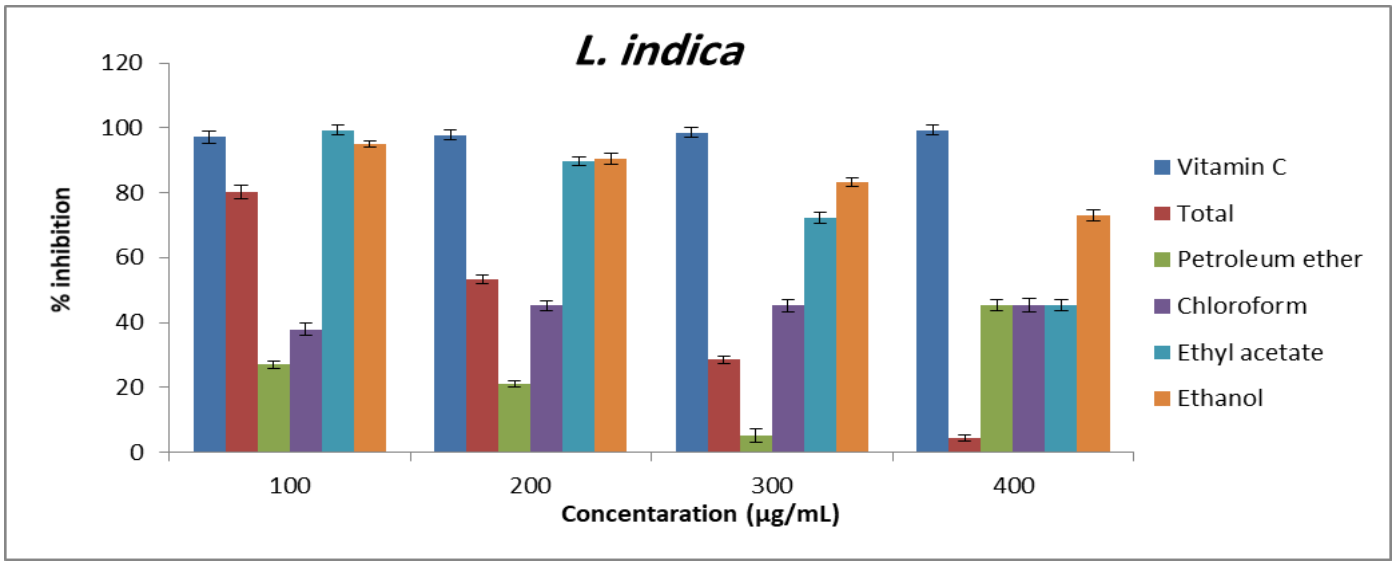

Figure 1. *In vitro* antioxidant activity of *L. indica* different extracts using DPPH method and vitamin C as a standard.

Petroleum ether recorded the highest inhibition percent at 400 µg/mL (45.20 %), while significant decrease in inhibition percent at 100, 200 and 300 µg/mL, was observed. In addition, significant decrease was noticed at different concentrations of *L. indica* chloroform extract as compared to vitamin C (37.90, 45.20, 45.20 and 45.20 %, at 100, 200, 300 and 400 µg/mL respectively). Moreover, ethyl acetate and ethanol extracts showed insignificant change at 100 and 200 µg/mL as compared to vitamin C, while 300 and 400 µg/mL exhibited significant decrease (72.26, 45.25 ,respectively, for ethyl acetate and 83.20 and 72.99 %, respectively, for ethanol extract). It could be concluded that the highest percentage of inhibition is recorded for ethyl acetate and ethanol extracts as compared to vitamin C.

For *S. splendens*, Fig. 2 showed significant decrease in inhibition percentage of 100 µg of its total extract (70.8 %) as compared to the corresponding concentration of vitamin C (97.08). While, at 200, 300 and 400 µg, the inhibition percentages decreased as compared to the relative concentrations of vitamin C. Petroleum ether extract of *S. splendens* showed the highest percentages of inhibition at 200, 300, 400 µg/mL reached to 43.00, 45.25 and 45.20 % , respectively as compared to vitamin C (97.80, 98.50 and 99.27 %, respectively) . More or less similar results were achieved for chloroform extract of *S. splendens*. On the other hand, ethyl acetate exhibited significant increase in inhibition percent at 100, 200 and 300 µg/mL (69.90, 63.50 and 42.30 %, respectively), while, significant decrease was detected in inhibition percent at 400 µg/mL (18.20 %). Insignificant changes in inhibition percent of *S. splendens* ethanol extract was observed at several concentrations as compared to vitamin C. Thus, it could be suggested that, ethanol extract declared the highest inhibition percent as compared to vitamin C followed by ethyl acetate extract.

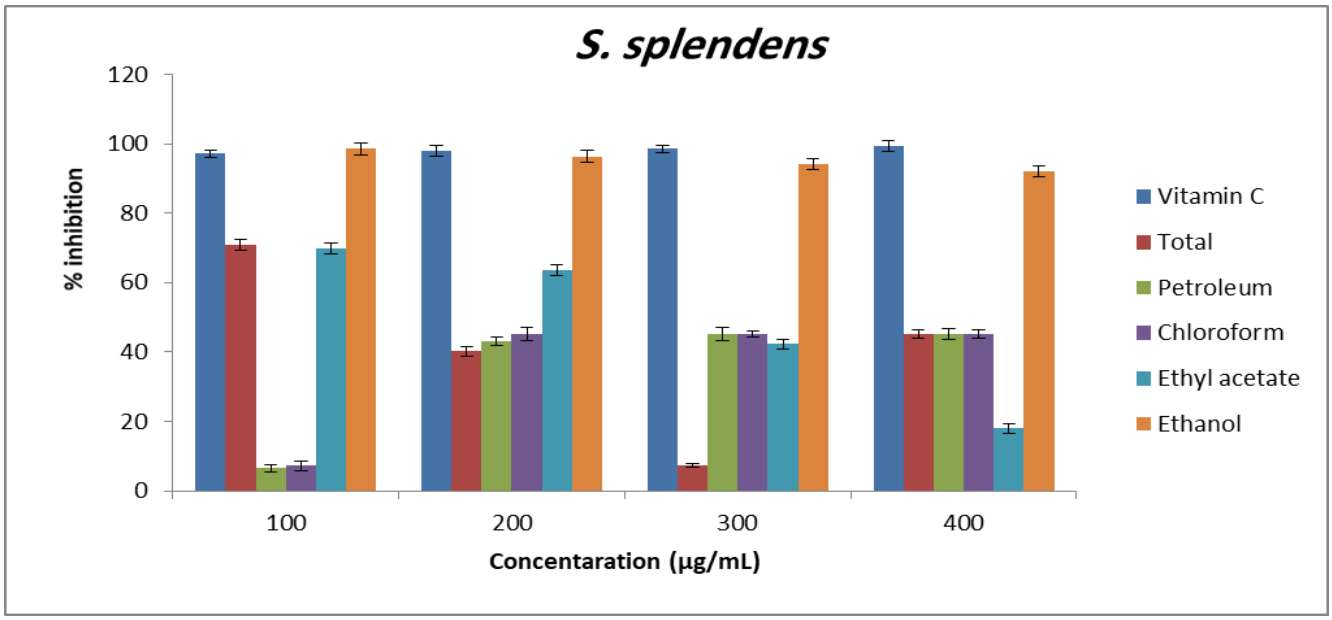

Figure 2. *In vitro* antioxidant activity of *S. splendens* different extracts using DPPH method and vitamin C as a standard.

3.3. *In vivo* antidepressant activity:

In vivo antidepressant-like effects of all extracts of both plants (100 mg/Kg) were shown in Table 2.

All groups of both plants and the standard (fluoxetine) showed significant decrease in immobile time (seconds) and increase in the ability of rat groups to move and swim for a longer time than the depressed control group (group 2)

For *L. indica*, depressed groups treated with either ethyl acetate extract (group 3) or ethanol extract (group 4) showed percent of immobility inhibition of 28.6 % and 9.6 %, respectively. For *S. splendens*, depressed groups treated with either ethyl acetate extract (group 5) or ethanol extract (group 6) showed percent of immobility inhibition of 32.5 % and 10.2 %, respectively. Where, fluxoteine drug (group 7) showed a bit higher percent of immobility inhibition (54.7 %).

3.4. Investigation of the phyto-constituents of the different extracts of the plants:

The physical and chemical properties of the different extracts of *L. indica* and *S. splendens* are presented in Table 3.

Table 2. Effect of different extract of *S. splendens* and *L. indica* and fluoxetine drug on the floating time in porsolt's forced swimming test in mice (n=6).

Group	Immobility time more than 6 minutes Mean ±S.E.	% Percent Inhibition
Group 1 (control normal)	**24.6±0.9**	**88.4**
Group 2 (control depressed)	**212.8±5.4**	**-**
Group 3 (*L. indica* ethyl acetate extract)	**151.9±6.3***	**28.6**
Group 4(*L. indica* ethanol extract)	**192.3±6.8***	**9.6**
Group 5 (*S. splendens* ethyl acetate extract)	**143.5±5.3***	**32.5**
Group 6 (*S. splendens* ethanol extract)	**191.2±6.7***	**10.2**
Group 7 (fluoxetine as standard)	**96.3±3.6***	**54.7**

***** Significantly different at $P < 0.05$ compared to control depressed.

Table 3. Physical and chemical characters of the different extracts of *L. indica and S. splendens*

Plant	***L. indica***		***S. splendens***	
Extract	**EAE**	**EE**	**EAE**	**EE**
Color	**Dark brown**	**Dark brown**	**Dark brown**	**Dark brown**
Condition	**Waxy**	**Waxy**	**Waxy**	**Waxy**
Carbohydrates and/or glycosides	+++	+++	+++	+++
Sterols and/or triterpenes	-	-	-	-
Flavanoids	+++	+++	+++	+++
Tannins	+++	+++	+	+
Anthraquinones	-	-	-	-
Alkaloids	-	-	-	-
Saponins	+	+	-	-
Coumarins	-	-	-	-

+++: Present in a high concentration, +: Present in a low concentration,-: Absent.

4. Discussion

Plants and organisms are used in a variety of ways in the production of conventional and alternative medicines. The beneficial active ingredient of the plant may be found anywhere in its physical structure, such as in the petal or stem of a flower.

Behavioral depression is a complication which leads to a variety of emotional and physical disorders and can decrease a person's ability to function properly at many activities.

Different extracts of *L. indica* and *S. splendens* aerial parts were prepared to test their activities as antioxidants and antidepressants. The yields of the different extracts of both plants were variable. For *L. indica*, the petroleum ether extract gives the highest yield followed by the ethanol extract, while the chloroform and the ethyl acetate extracts give a moderate yield.

Concerning *S. splendens*, the petroleum ether extract gives the highest yield followed by the chloroform and ethanol extracts then the ethyl acetate extract.

Oxidative stress has been associated in the patho-physiology of many neurological disorders.
Because of the strong correlation between oxidative stress and behavioral depression [20], all extracts of both plants were primarily subjected to be tested for their antioxidant activity *in vitro* using DPPH method to highlight the most active extracts to be injected *in vivo* to evaluate their antidepressant-like effect. Antioxidants are substances that can replace, delay or prevent oxidations of other substrates. Antioxidants may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species, which are linked to a variety of diseases, resulting in different behavioral and biochemical consequences. Antioxidants can perform their action by reducing oxygen concentration, capturing singlet oxygen, inhibiting first chain launch by scavenging initial radicals binding metal ion catalysts, degrading primary products to non-radical ones, and breaking of chains to avoid persistent hydrogen abstraction from substrates [21]. Several synthetic antioxidants such as, Butylated hydroxy anisole (BHA) and Butylated hydroxy toluene (BHT) are commercially available but are quite unsafe and their toxicity is a problem of concern. So, identifying natural sources with high antioxidant ability may be considered a main route for controlling many complications. Anthocyanins, phenolics and flavonoids are considered as natural, safe and bioactive antioxidants [22].
All extracts of both plants were tested for their DPPH free radical scavenging activity *in vitro* using serial concentrations between 100- 400 µg/mL and ascorbic acid as a standard.
For *L. indica*, its total, ethyl acetate and ethanol extracts showed the highest DPPH radical scavenging activity. These results might come with Moussa et al. (2011), where the chloroform and methanol leaf extracts of *L. indica* at 50 µg/mL were tested for their antioxidant activity using the DPPH assay. The results were expressed as percentage inhibition of free radical scavenging activity of the chloroform and methanolic extracts of the *L. indica* analyzed. The values ranged from 0.5 to 49% and from 3 to 96% for chloroform and methanolic extracts, respectively [23]. The variation of the free radical scavenging activity may be due to the differences in their secondary constituents [24]. However we should keep in mind that the high DPPH scavenging activity value obtained for ascorbic acid is because we performed the assay on highly purified reference standard and not in complex materials such as that of plant species analyzed. This implies that although there are plants with good antioxidant abilities, further concentration or purification is needed to achieve better antioxidant capacities.
In accordance with the present results, Lee et al. (2014) suggested that *L. indica* has a great potential as a cosmeceutical raw material as well as antioxidant, anti-inflammatory and collagenase inhibition activity [25]. According to DPPH scavenging radical activity, acetone extract of *L. indica* branch was higher than 73% at the 50 ppm concentration. The decrease in absorbance of DPPH (increase in inhibition percent) caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a color change from purple to yellow. A lower value of EC50 indicates a higher antioxidant capacity.
For *S. splendens*, the results indicated that the total methanol extract had a notable effect on scavenging of DPPH free radical between 100- 400 µg/mL when compared with ascorbic acid. The present results are in line with Kumar et al. (2010), who reported that, the methanolic extract of *S. splendens* has high antioxidant activity due to the presence of phenolic and flavonoid compounds [26]. Other polar extracts prepared with either ethyl acetate or ethanol also showed a higher antioxidant activity. Thus, the antioxidant and free radical scavenging activities of polar extracts of *S. splendens* in the present study might be because of the occurrence of high amounts of phenolic and flavonoid mixtures. In parallel results, Neagu et al. (2014) declared the existence of a correlation between the polyphenols content (1.04 – 1.72 mg/mL) and antioxidant activity [27]. In a good concomitant with the present findings, Narayan and Mittal (2015), investigated the *in vitro* antioxidant action of *S. splendens* roots' differenet extracts (petroleum ether, ethyl acetate and methanolic extracts) and they found that these extracts may have high antioxidant action [28].
Oxidative stress can influence brain functions and may lead to several neurodegenerative diseases [29]. Depression is a complex disorder and the hypothesis underlying its pathogenesis is still unknown. The forced swim test is the most usually engaged behavioral model. Although this behavioral model does not mimic the human state of major depression, it is used to screen antidepressants molecules. Reserpine injection leads to high oxidative stress induction in brain rats that caused behavioral depression in the diseased groups. Reserpine injection (0.25 mg/kg s.c) every 48 hours for 20 days, resulted in depressive-like behaviors in rat models, while significant reduction in depressive-like behaviors was evident in the diseased rats treated with poly phenolic-rich extracts and fluoxetine (positive control). For *L. indica* and *S. splendens*, all polar extracts and the standard fluxoteine showed significant differences (at $p<0.05$) when compared to the control depressed group but the ethyl acetate extracts showed a bit higher percent of inhibition with a lower time for that rats' immobility in the water tank. To date, this is the first study to evaluate the antidepressant-like effects of *L. indica* and *S. Splendens*.
Pythochemical screening of both plants were performed to determine the main major class of compounds that may be responsible for the biological activities. For both plants, results revealed that extracts prepared with polar

solvents being rich in carbohydrates, flavonoidal contents and tannins. Plant derived-flavanoids and phenolic compounds have established significant care due to their different biological activites like being, antioxidant, anti inflammatory, antitumor as well as having lower toxicity when compared to synthetic ones such as, butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate. The systematic literature collection, pertaining to the present investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Flavanoids, one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties [30]. Latest studies have approved that, various dietary polyphenolic compounds extracted from plants, are extra active as antioxidants *in vitro* than vitamins C or E, and so this might contribute to the protecting actions *in vivo* [22]. Nowadays, *In vitro* antioxidant studies are broadly carried out to display different plants containing phenolic and flavanoids compounds. Significance difference of either antioxidant or antidepressant-like activities among extracts may be contributed to the occurrence of phenolic constituents among extracts. So, picking the suitable solvent is one of the greatest issues for attaining extracts with high amount of bioactive mixtures [31].

Conclusions and Recommendations

S. splendens and *L. indica* can be used to control behavioral depression but more research is needed in determining the exact mechanism.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

This work was supported by the National Research Centre internal project no [grant number 10010320].

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