Preliminary Phytochemical, Acute Oral Toxicity and Anticonvulsant Activity of the Leaves of *Solanum Nigrum* Linn.

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Abstract: The purpose of this research is to investigate a preliminary phytochemical, acute oral toxicity and anticonvulsant activity of the leaves of *Solanum nigrum* Linn. Qualitative analysis for phytochemicals was carried out. Acute oral toxicity test was conducted. The anticonvulsant effect of ethanolic extract from the leaves of *Solanum nigrum* on pentylenetetrazole (PTZ) - induced seizures in male mice was examined. Phytochemical screening revealed that the leaves of *Solanum nigrum* contain carbohydrates, flavonoids, saponins, tannins, alkaloids, phenols and steroids. The oral median lethal dose of the extract was estimated as upper 5000 mg/kg. In PTZ-induced seizures, the extract significantly delayed the latency of convulsant, reduced the recurrence of convulsant and provided significant protection against death. These findings suggest that the ethanolic leaf extract of *Solanum nigrum* is safe and possess anticonvulsant activity in PTZ-induced seizure mice. These predictors, however, need further work to validate reliability.
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1. Introduction

Medicinal plants play a significant role among the traditional and modern systems. Their use has been multiplied through various researches and application due to a number of side effects from the use of synthetic drugs, antibiotics and high cost. The people of rural area are mainly depending on the traditional medicine for curing their ailments because of the non-availability of modern medicines and hospitals. In developing countries, 80% of the population still use traditional folk medicines obtained from natural resources (N.R. Farnsworth,O. Aereele and A.S. Bingel,1985).

Solanum nigrum (*S. nigrum*) is a widely distributed tropical plant. It is a medicinal plant used to treat a wide range of disorders including epilepsy. The plant has been reported to have antiperiodic, antiphlogistic, diaphoretic, diuretic, emollient, febrifuge, narcotic, purgative and sedative (Plant for a future, 2013). Epilepsy is a brain disorder in which clusters of nerve cells, or neurons, in the brain sometimes signal abnormally. Neurons normally generate electrochemical impulses that act on other neurons, glands, and muscles to produce human thoughts, feelings, and actions. In epilepsy, the normal pattern of neuronal activity becomes disturbed, causing strange sensations, emotions, and behavior, or sometimes convulsions, muscle spasms, and loss of consciousness. During a seizure, neurons may fire as many as 500 times a second, much faster than normal. In some people, this happens only occasionally; for others, it may happen up to hundreds of times a day (Medicine Net, 2013).

Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing countries is 100 per 100,000 (Epilepsy: Etiology, epidemiology and prognosis, 2012). According to the report on epidemiology, in Viet Nam, epilepsy occupies 0.44 – 0.55% of population. However, only 29/189 patients are treated periodically with antiepileptic drugs (AEDs). The missed treatment of epilepsy in Viet Nam is very high (84.7%) (Sức khỏe và Đời-sống, 2013).

Modern AEDs are complicated by the inability of drugs to control seizure in some patients and adverse effects such as absence of hypersensitivity reactions, weight problems, and drug interactions that cause central nervous system toxicity (Dieter Schmidt, 2009). It has been observed that the presently available AEDs are unable to control seizures effectively in as many as 25% of the patients (Mattson R.H, 1992). The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate carry with them several serious side effects notably neurotoxicity (Gupta Y.K, Malhotra J., 1997). As majority of AEDs are consumed life long, co-administration of other drugs predisposes to the risk of drug interaction. Thus, it is necessary to investigate new antiepileptic agent that is significantly potential in epileptic treatment as well as safe in terms of toxicity. The target of treating epilepsy is not only to prolong the latency or reduce the recurrence of convulsions but also to lead a self-sustained life.

Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to possess promising anticonvulsant activities in animal models for screening for anticonvulsant activity (Sandabe UK, Onyelili PA, Chibuzo GA., 2003; Ahmad B, NaeemAK, Ghufran A. 2005; Ma Eva G-T, Elisa T, Leonor L-M, Andrés N, Adelfo R-R, Adrián M. 2006; Salahdeen HM, Yemitan OK. 2006; John AOO. 2007) and can be a source of newer and safer anticonvulsants.

2. Material and Methods

2.1 Plant materials

Leaves of *S. nigrum* were collected from a mountain in KrôngNô, ĐắkNông Province of Viet Nam in August, 2012. The plant was identified by Associate Professor Dr. Tran Van Minh of the Institute of Tropical Biology, Viet Nam. The specimen was deposited in the herbarium of Applied Biochemistry Laboratory, Department of Applied Chemistry, School of Biotechnology, International University, Viet Nam National University-Ho Chi Minh City, Viet Nam with voucher No. HB-BIO-10-08-2.

2.2 Extraction

Fresh leaves of *S. nigrum* were dried in and ground into fine powder and referred to as powdered leaves. The powdered leaves (25 g) were defatted with 350 mL of 70% ethanol (60-80°C) in Soxhlet apparatus. The extract was then evaporated in vacuum to give a brownish residue. The residue, subsequently referred to as the extract, was stored in a refrigerator until required for further use.

2.3 Experimental animals

Healthy Swiss mice *Mus musculus var*. Albino weighing 25-30 g were procured from Pasteur Institute of Ho Chi Minh City. They were housed in clean cages and had free access to standard pallet diet and water ad libitum. During the experiment, mice were placed under a controlled environmental condition with 12 h of light and dark cycle. All the animals were acclimatized to laboratory conditions for a week prior to commencement of experiments. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were conducted in accordance with animal use ethics as accepted internationally.

2.4 Phytochemical screening

Phytochemical examinations for extracts were performed using standard procedures (Harbone JB., 1984).

2.5 Oral acute toxicity test

Acute toxicity of the plant extract was carried out *in vivo* in healthy female albino mice weighing 25-30 g, labeled individually. Prior to dosing, mice were fasted overnight and the dose for each mouse was determined based on the body weight. Solutions of the dried extracts were prepared using distilled water. The study was conducted as per Organization of Economic Cooperation and Development (OECD/OCDE) Test guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme-AOT425StatPgm, version 1.0. Up and Down and classified based on the provisions of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) as adopted by the United Nations Economic and Social Council in July 2003.

2.6 Anticonvulsant activity methodology

Mice were randomly divided into six groups of five mice each (n=5). Group I served as control received equivalent amount of the distilled water; group II, III, IV, and V received the ethanolic leaf extract of *S. nigrum* of the dose of 50, 100, 200 and 300 mg/kg, *p.o,* respectively; and group VI received phenobarbital (100 mg/kg *p.o*) and was served as reference standard.

All the leaf extracts and standard drugs were administered 60 min before the administration of PTZ (85 mg/kg *i.p*) and the mice were observed for the convulsions. Mice were immediately placed individually in a cage, observed and counted to determine the latency and the recurrence of convulsions for each mouse. Values were expressed in terms of mean ± SEM. Manifestations of the seizures were rated on a 6-point scale according to the Racine's scale (1972) that is widely used in studies on animal models of epilepsy (Table 1) (Setkowicz Z., K. Klak and K. Janeczko, 2003;Turski, W.A., E.A. Cavalheiro, M. Schwarz, S.J. Czuczwar, Z. Kleinrok and L. Turski, 1983).

Table 1. 6-point scale for anticonvulsant activity

Light seizures	Intermediate seizures	Heavy seizures
0.5: Immobility, piloerection, salivation, narrowing of eyes, face and vibrissae twitching, ear rubbing with forepaws	**1.5**: Clonic movements of forelimbs and mild whole body convulsions, exophthalmia, aggressive behavior	**2.5**: Rearing and falling, eye congestion
1.0: Head nodding and chewing movements	**2.0**: Rearing and running with stronger tonic-clonic motions including hind limbs, tail hypertension, lock jaw	**3.0**: Loss of postural tone with general body rigidity

2.7 Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS for windows, version 16). The variability degree of the result is expressed as mean ± the standard error of the mean (Mean ± SEM). The significance of the difference between samples was determined using Turkey HSD test. The difference was regarded significant when $p < 0.05$ and non-significant when $p > 0.05$, where P is a level of significance.

3. Results

3.1 Phytochemical screening

Phytochemical screening of the ethanolic leaf extract of *S. nigrum* revealed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, phenols, and steroids (Table 2).

3.2 Oral acute toxicity test

The LD50 value of the ethanolic leaf extract of *S. nigrum* was found to be greater than 5000 mg/kg of body weight. This study shows that the leaf extract of *S. nigrum* is safe or non-toxic even in high acute doses.

3.3 Anticonvulsant activity

The effect of ethanolic leaf extract of *S. nigrum* on PTZ-induced seizures in male mice is showed in Table 3.

Table 2. Phytochemistry of the ethanolic leaf extract of *S. nigrum*

Phytochemical constituents	Phytochemical test	Inference
Carbohydrates	Barfoed's test	+
Flavonoids	Hexane Conc. HCl and Magnesium ribbon	+ +
Saponins	Emulsion test Frothing test	+ +
Tannins	Ferric chloride test Diethyl ether	+ -
Alkaloids	Dragendorff's test Mayer's test Chloroform	+ + -
Phenols	Diethyl ether Ethyl acetate	+ +
Steroids	Ethyl acetate Methanol	+ -

"+": positive test result, "-": negative test result.

Table 3. Effect of ethanolic leaf extract of *S. nigrum* on PTZ-induced seizures in male mice

Group (n=5)	Latency of convulsant (second)	Recurrence of convulsant (time)	No. of convulsant	No. of death	Protection (%)
I	330 ± 25.994	16.0 ± 1.14	5	5	0%
II	344.80 ± 305.017	5.4 ± 2.302	5	5	0%
III	580.20 ± 279.556	3.0 ± 1.414	5	3	40%
IV	686.80 ± 269.487	2.0*± 1.732	5	1	80%
V	721.40 ± 154.407	0.8*± 0.447	4	0	100%
VI	0	0	0	0	100%

*Each value represents mean ± SEM; *$p < 0.05$ compared with the control

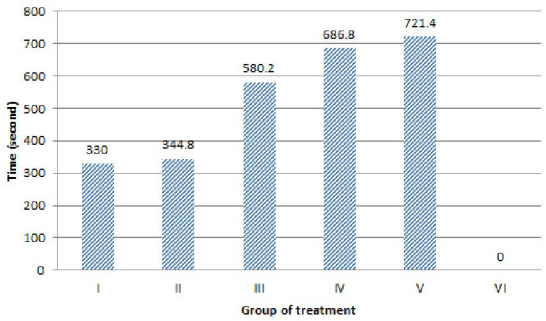

Figure. 1. Effect of different doses of ethanolic leaf extract of *S. nigrum* (50, 100, 200, 300 mg/kg) on the latency of convulsions in mice.

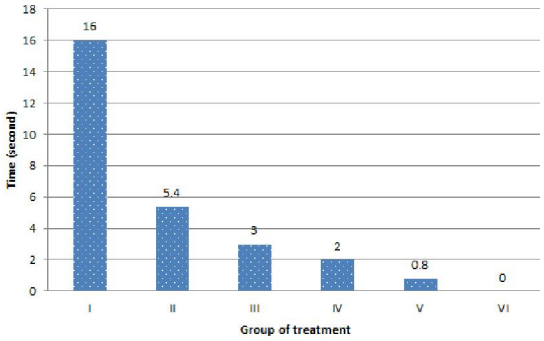

Figure. 2. Effect of different doses of ethanolic leaf extract of *S. nigrum* (50, 100, 200, 300 mg/kg) on the recurrence of convulsions in mice.

The ethanolic leaf extract of *S. nigrum* significantly delayed the latency of convulsions ($p < 0.05$) in a dose-dependent manner in PTZ-induced seizure mice. The results are shown in Table 3 and Figure 1 for PTZ-induced seizure, the latency of convulsions in group I (control) was 330.00 ± 25.994 seconds. In group IV and V, 200 and 300 mg/kg of the extract markedly prolonged the latency of seizures to double as compared to that of group I.

Notably, the results are shown in Table 3 and Figure 2 expressed a rapid decline in the recurrence of convulsions by seconds. In group I, convulsion in tested mice was lasted for 16.0 ± 1.14 seconds. This number decreased dramatically to 5.4 ± 2.302 in group II and continued to decrease but more steeply to 0.8 ± 0.447 in group V. There were slight convulsions in mice of group V, but they were neglectable.

In conclusion, all the animals in group I died while in group V, at the dose of 300 mg/kg of body weight, the mortality was 100%. This is comparable to the values obtained in group VI (phenobarbital) where 100% mortality was recorded. In group IV, the protection yielded 80% indicating a potential anticonvulsant activity of the leaf extract at lower concentration. However, the predictions are needed to test for the reliability and validating.

4. Discussions

The *S. nigrum* is an annual plant, common and generally distributed in the high land of Vietnam. It is one of the most cosmopolitan of wild plants, extending almost over the whole globe.

The aim of this study was to evaluate the anticonvulsant effects of the ethanolic leaf extract of *S. nigrum*. The results obtained here show that the extract significantly delayed the latency of seizures, reduced the recurrence of seizures and provided significant protection against death.

PTZ exert convulsant effect by inhibiting the activity of gamma amino butyric acid (GABA) at $GABA_A$ receptors (DeSarro A, Cecchetti V, Fravolini V, NaccariF, Tabarrini O and DeSarro G, 1999). GABA is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively (Gale K., 1992).

Phytochemical screening carried out in the present study shows that the extract contains flavonoids. Is is believed that the medicinal plant-contained flavonoids might play a key role in anticonvulsant activity (Chauhan A.K, Dobhal M.P, Joshi B.C, 1988; Giulia, Di Carlo, Nicola Mascolo, Angelo A, Izzo and Fracesco Capasso, 1999). Flavonoids, an important class of natural compounds, have demonstrated the activities on central nervous system such as affinity for $GABA_A$ receptors and anticonvulsion effects (Miliauskas G, PR V and Van Beek T.,2004; Huen M, Leung J, Ng W, Lui W, Chan M, Tze-FeiWong J and Xue H, 2003).

In conclusion, considering the recorded effects of ethanolic leaf extract of *S. nigrum* in the present study, further research on the effect of this plant against convulsion should be investigated.

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References

1. N.R. Farnsworth, O. Aereele and A.S. Bingel (1985), Medicinal Plants in therapy, Bulletin of the World Health Organization,63: 965-981
2. *Solanum nigrum* - L. (2013). Plant For A Future, available from: http://www.pfaf.org
3. Government. Epilepsy (Seizures Disorders) (2013),available:http://www.medicinenet.com/seizure/article.htm
4. WHO. Epilepsy: Etiology, epidemiology and prognosis (2012), available from: www.who.int/entire/mediacentre/factsheets/fs165/en/
5. WHO cam kết hỗ trợ Việt Nam phòng chống bệnh động kinh (2013). Sức khỏe và Đời sống, available from: http://suckhoedoisong.vn/20130719093934733p61c67/who-cam-ket-ho-tro-viet-nam-phong-chong-benh-dong-kinh.htm
6. Dieter Schmidt. (2009) Drug treatment of epilepsy: Options and limitations. Epilepsy & Behavior. 15: 56–65.
7. Mattson R.H. (1992). Drug treatment of partial epilepsy. Adv Neurol. 57: 643-650.
8. Gupta Y.K, Malhotra J. (1997) Adenosinergic system as an endogenous anticonvulsant mechanism. J Physiol Pharmacol. 41: 329-343.
9. Sandabe UK, Onyelili PA, Chibuzo GA. (2003). Sedative and anticonvulsant effects of aqueous

extract of *Ficus sycamorus* L. (Moraceae) stembark in rats. Vet Archiv. 72(2):103–110
10. Ahmad B, Naeem AK, Ghufran A. (2005). Innamudin Pharmacological Investigation of *Cassia sophera*, Linn. *Var. purpurea*, Roxb. Medical Journal of Islamic World Academy of Sciences. 15(3):105–109.
11. Ma Eva G-T, Elisa T, Leonor L-M, Andrés N, Adelfo R-R, Adrián M. (2006). Anticonvulsant Effect of *Annona diversifolia* Saff. And Palmitone on Penicillin-induced Convulsive Activity. A Behavioral and EEG Study in Rats. Epilepsia. 47(11):1810–1817.
12. Salahdeen HM, Yemitan OK. (2006). Neuropharmacological Effects of Aqueous Leaf Extract of *Bryophyllum Pinnatum* in Mice. African Journal of Biomedical Research. 9(2):101–107.
13. John AOO. (2007). Anticonvulsant effect of *Sclerocarya birrea* (A. Rich.) Hochst. Subsp. *caffra* (Sond.) Kokwaro (Anacardiaceae) stem-bark aqueous extract in mice. Journal of Natural Medicines. 61(1):67–72.
14. Harbone JB. (1984). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 2nd Ed., Chapman and Hall, London. 84-274.
15. Setkowicz Z., K. Klak and K. Janeczko.(2003). Long term changes in postnatal susceptibility to pilocarpine induced seizures in rats exposed to gamma radiation at different stages of prenatal development. Epilepia, 44: 1267-1273.
16. Turski, W.A., E.A. Cavalheiro, M. Schwarz, S.J. Czuczwar, Z. Kleinrok and L. Turski. (1983). Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. Behav. Brain Res., 9(3): 315-335.
17. Miliauskas G, PR V and Van Beek T. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. J. Food Chem, 85: 231-237.
18. Huen M, Leung J, Ng W, Lui W, Chan M, Tze-Fei Wong J and Xue H.(2003). 5, 7- Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from Scutellaria baicalensis Georgi, with selective antagonistic properties. J. Biochem. Pharmacol. 66: 125-132
19. Chauhan A.K, Dobhal M.P, Joshi B.C. (1988). A review of medicinal plants showing anticonvulsant activity, Journal of Ethnopharmacology, 22, 11–23.
20. Giulia, Di Carlo, Nicola Mascolo, Angelo A, Izzo and Fracesco Capasso. (1999). Flavonoids: oldand new aspects of a class of natural therapeutic drugs. Life sciences, 1999, 65(4),337-353.
21. De Sarro A, Cecchetti V, Fravolini V, Naccari F, Tabarrini O and De Sarro G. (1999). Effects of novel 6-desfluoroquinolones and classic quinolones on pentylenetetrazole-induced seizures in mice, Antimicrobial Agents and Chemotherapy, 1999, 43,1729–1736.
22. Gale K. (1992). GABA and epilepsy: basic concepts from preclinical research, Epilepsia, 1992, 33,S3–S12.

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