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Phytochemical screening and potential anticonvulsant activity of aqueous root extract of *Decalepis nervosa* Wight & Arn.

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ABSTRACT

The effect of phytochemicals in the aqueous root extracts of *Decalepis nervosa* Wight & Arn. (ARDN) (Family: Apocynaceae) was investigated on an anticonvulsant action in laboratory animals (mice). The DN roots were extracted using microwave extraction method with aqueous solvent using two parameters, such as temperature variation and extraction time. The phytochemical screening followed by thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) studies was performed. The pentylenetetrazole and isoniazid models were used with two doses, 250 mg and 500 mg, for the anticonvulsant effects of the ARDN extract. The microwave extraction during 15 min at 60°C temperature revealed the DN root aqueous extract yielded higher (36.20%) than other selected parameters. The phytochemical screening study indicated the existence of alkaloids, flavonoids, glycosides, and phenols, and further TLC, HPTLC studies confirmed the presence of Quercetin and Gallic acid that demonstrated a strong dose dependent anticonvulsant activity. The other biochemical parameters such as lipid peroxidation level showed significant dose-dependent inhibition in malondialdehyde content (29.86%) in mice brain, and also, glutathione content showed significant dose-dependent increased in percentage content (58.22%) in brain of mice. Finally, ARDN's potential anticonvulsant was first established through the optimized microwave extraction method with the presence of polyphenoilc phytoconstituents.

Keywords: Anticonvulsion, Biochemical estimation, Correlation, *Decalepis nervosa*, High-performance thin-layer chromatography

INTRODUCTION

Epilepsy is the most serious neurological condition as chronic brain damage that cannot be transmitted. This is also known as seizure and in a community of brain cells that it is the product of repeated electrical discharges. It ranges from the sudden muscle jerks to extreme and prolonged convulsions, resulting in loss of control or consciousness, and speech, vision, hearing and mood, or other cognitive functions. Up to 10% of people are reported to have one seizure during their lifetime.[1] There are many prescription drugs available on the market to treat this epilepsy, but few of them are very relevant, namely, gabapentin, tiagabine, topiramate, vigabatrin, lamotrigine, and zonisamid. All these medications are successful in the short term and still do not cure root-level epilepsy. Nevertheless, these medicines have some undesirable side effects, namely, teratogenicity, chronic toxicity, cognitive, and behavioral changes which can also be observed.[2,3] The conventional medicine method therefore plays as important role as alternative treatment of this epileptic condition. The conventional medicine program, according to research evidence, helps to treat epilepsy and associated disorders such as insomnia, anxiety, hallucinations, dizziness, headaches, migraines, pains, and schizophrenia.[4-6] Therefore, herbal medicine applications are gaining popularity in treating epileptic seizures, likely due to less expensive side effects and contraindications. These herbal medicinal products are derived from chemical constituents that occur naturally in different plant parts which play a key role in controlling the disorder.

Of late, *Decalepis nervosa* (DN, family: Apocynaceae) is one of the major climber shrub species of genus "*Decalepis*"

which is located in Tamil Nadu (Western Ghats regions and Nilgiri region), Kerala, and Karnataka but due to over exploitation and human causes the selected said that plant becomes endangered in India. The plant is commonly referred to as known as a nerved leaf, Swallow root, etc.[7] As with other *Decalepis* species, DN does not have much scientific data on particular monographs as well as therapeutic applications. In general, roots of other tuberous *Decalepis* species such as *Decalepis arayalpathra*, *Decalepis Hamiltonii,* and *Decalepis salicifolia* are fleshy and sweet vanilla like odour. These roots are widely used as appetite stimulant, to sooth flatulence and as tonic.[8] Since of the sweet vanilla essences, these roots are often used as flavoring principle,[9] and in preparation of pickles,[10] and as food preservative, preparation of nutraceuticals and various pharmaceutical products.[11] Conventionally, the root possesses potential antimicrobial, antidiabetic, antioxidant, antiinflammatory, chemoprotective, cytoprotective, insecticidal, neuroprotective (against epilepsy and central nervous system disorders), and hepatoprotective properties.[11] These activities are due to presence of essential phytoconstituents that possess key role in therapeutic efficacy. Among them 2-hydroxy-4-methoxy benzaldehyde is present in huge quantity, followed by other phenols, flavonoids, volatile oils (vanillin, benzyl aldehyde, salicylaldehyde, methyl salicylate, benzalcohol, 2-phenylethyl alcohol, and ethyl salicylate), etc., which show significant medicinal properties,[12,13] but no such scientific evidences related to the root morphology or chemistry or any therapeutic efficacy on *D. nervosa*.[14] The plant genus *Decalepis* has species name nervosa which may indicate activity on nerve, and thus, anticonvulsant activity has been investigated based on the species name of the present research. The detailed phytochemical studies, as well as the yield effect of extraction and its correlation with the said therapeutic effect with the major chemical constituent described. This investigation was the first study on the therapeutic efficacy of DN root extract that helps in several activities further.

MATERIALS AND METHODS

Collection of DN Roots and Its Processing

DN roots were collected by Dr. Gokul Sivaraman, National Post Doctoral Fellow, CIMAP Research Centre, Allalasandra, GKVK Post, Bangalore, from Western Ghats region of Tamil Nadu and procured from him for this present study in 2018 and the same was authenticated by Dr. Rajasekharan P E, Principal Scientist, Department of Plant Biotechnology, Indian Institute of Horticultural Research, Bengaluru, India. The roots were preserved in Pharmacognosy laboratory as herbarium (Herbarium numbers: KD-DNR/KCP-128/2018) [Figure 1].

The roots were washed and dried under shade for 7 days then coarsely ground with small hammer mill. To protect against moisture and for further use, the powdered DN roots were kept in sealed plastic cover. Microwave extractor used aqueous solvent for extraction.

Extraction of DN Root

Coarsely dried DN root powder (20 g) was separately suspended in a 250 ml Teflon extraction vessel in 100 ml of aqueous solution. The vessel was put in the microwave system and extracted at various temperatures (20°C, 40°C, 60°C, and 80°C) with varying extraction time (5, 10, 15, and 20 min, respectively) at constant voltage of 500 W. The vessel was allowed to cool at 25°C then filtered with Whatman filter paper and dried by rotary evaporator (at 45°C) to obtained extract and yield for each cycle was measured. For further testing, all the extracts were stored in small glass bottles under cooling (4°C).

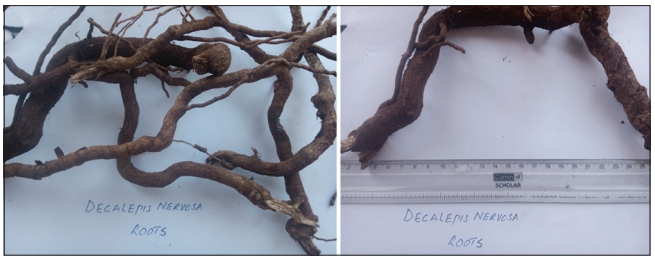

Figure 1: DN fresh roots

Phytochemical Screening

The presence of group of chemical components in the extract (extract with higher yield) was qualitatively screened by various chemical tests using the standard methods described in literatures.[15,16]

Experimental Animals and Group Selection

Healthy mice of either sex (Swiss albino stain with 25–35 g weight) have been selected after approval sanctioned by the Institutional Animal Ethical Committee (Ref No: KCP/PCOL/14/2018) for this research. They were procured from the in house animal house of Krupanidhi College of Pharmacy, Bangalore, India. The selected female mice were based on a regular period of the estrous. Hygienic polypropylene cages and under usual laboratory conditions with 1 week regular diet and water *ad libitum* were maintained until the experiments started. Constant temperature (25 ± 2°C) was maintained for all the mice (groups of six per cage) with 50% humidity and proper 12 h day and night cycle.

Acute Toxicity Study

Acute toxicity analysis for aqueous root extract of *Decalepis nervosa* (ARDN) root was performed as per the OECD guideline 423. Until dosing, the animals were fasted and the ARDN single dose was administered orally in the next day, as per each animal's body weight. The dose was prepared after the extract has been dissolved in distilled water. The single dose of extract (2000 mg/kg b.w) was initially administered to one animal and then monitored for any changes in behavior every 1 h, alternating for a whole day. Based on the positive response, the same dosage was given to another three animals, and behavioral changes were observed and reported for 7 days. The same way the experiment was replicated with the 5000 mg/kg body weight dose and observed again for 7 days and the results were reported.

Anticonvulsant Screening

Pentylenetetrazole (PTZ)-induced convulsion

Mice were grouped in to four, randomly divided into four groups (each group had n = 8 mice of either sex). Group I received respective vehicles; Group II for standard diazepam drug (2 mg/kg, intra-peritoneal [*i.p*]) and Group III, and IV treated with varying doses of ARDN. Statistically, all groups were compared with control group. Groups treated with control and extracts were fed orally, and regular *i.p*. medications were administered before seizures were caused. The extract dose was administered for 7 days and PTZ 80 mg/kg was injected subcutaneously (*s.c*) in mice 30 min after vehicle or extract and 30 min after the standard drug on the experimental day. Hind limb extension was taken as serve convulsion. Eventually, following observations such as seizure onset, seizure period (number of mice exhibiting seizures), and mortality duration of 30 min were reported.[17-19]

Isoniazid (INH)-induced convulsion

Mice were grouped into four (n = 8 mice of either sex in each group). Group I received respective vehicles, Group II was allotted for standard drug (diazepam 10 mg/kg, *i.p*). Group III and IV were treated with varying doses of ARDN. The extracts were administered as per the body weight of the mice by oral route after diluted in distilled water. Mice were given extracts for 7 days and INH 300 mg/kg was *s.c* injected to mice 45 min after vehicle or extracts and 30 min after the standard drug on the experimental day. Afterward, all the mice were tested for seizure, seizure occurrence, and death.[20]

Biochemical Estimation

Tissue preparation

Decapitation sacrificed mice of all the groups and the brain was isolated, homogenized in 0.9 percent sodium chloride using Remi motor (RQT-1.2.7A) for further biochemical estimation.

Lipid Peroxidation Estimation in Brain

1 ml of the medium suspension was taken from 10% homogenous tissue. Added 2 ml of 30% of trichloroacetic acid followed by 2 ml of thiobarbituric acid (0.8% TBA). At 80°C, the tubes were held for 30 min in the water bath. Then, the tubes were taken out and kept in ice cold water followed by centrifuged at 3000 rpm for 30 min. The absorbance of the supernatant was recorded at 530 nm against blank (2 ml distilled water, 2 ml of 30% TCA, and 2 ml of 0.8% TBA).[21] Ultimately, the contents of malonaldehyde (MDA) (nmoles/ml of protein in the tissue) were determined by:

$$\text{Concentration} = a \times (v/e) \times p$$

Where,
a =total solution in volume;
e= extinction coefficient ($1.56 \times 105 m^{-1} cm^{-1}$);
p = content of tissue protein (mg/g of tissue).

Estimation of Brain Glutathione (GSH)

0.02 M EDTA (2.5 ml) and 10% homogenate (2 ml, prepared in saline solution) were mixed and from which 2 ml of the mixture was taken, to that 4 ml of cold distilled water and 1 ml of 50% trichloroacetic acid was added. The mixture solution was, then, centrifuged at 3000 rpm for 20 min and from which few ml of the supernatant (2 ml), tris buffer (0.4M, pH 8.9) and few drops of 0.01M DTNB were mixed, and finally, the absorbance was recorded at 412 nm against distilled water as blank.[22] The GSH content was expressed as:

$$\mu \text{ mol/mg wet tissue: } [A/13600] \times \text{dilution factor} \times 1000$$

TLC Study

Thereafter, based on group of constituent present, TLC was performed for identification and separation of the constituent present which is responsible for the anticonvulsant activity. Various solvent systems were used for identification of main phytoconstituents present in the DN root extract (for extract with higher yield).

High-Performance Thin-layer Chromatography (HPTLC) Study

Based on TLC chromatogram, an aqueous extract, further, quantified using HPTLC using standard Quercetin (purity= 98%) and Gallic acid (purity = 97%).

Chromatographic Condition

Samples are applied on precoated silica gel 60 F_{254}TLC plates (20 × 10 cm) by Linomat V sample applicator. The sample volume is applied in 4.0 µl each as 5 mm band length in 3 × 10 silica gel. Reprostar Chromatography Documentation Apparatus is used for photographs of the HPTLC plates.

Detection and Quantification

The layers of sample and standard were developed, with mobile phase (toluene: ethyl acetate: formic acid, 7:5:1, v/v), in a chamber that was previously saturated at room temperature with mobile phase vapor. After removal of plates from chamber, completely dried in air at room temperature and peak areas for samples and standard were recorded by densitometry in absorbance/reflectance mode at λ max = 254 and 366 nm, by means of a CAMAG TLC Scanner 3 with win CATS version [3.2.1].[23]

Stock Solution of Gallic Acid

Gallic acid (10 mg) was dissolved in methanol and made up to 10 ml volume to yield a concentration of 1 mg/ml.

Stock Solution of Quercetin

Quercetin (10 mg) was dissolved in methanol and made up to 10 ml volume to yield a concentration of 1 mg/ml.

Correlation Study

Yield of the extract was correlated with the microwave extraction method using time and temperature parameters. Thereafter, percentage yield was correlated with amount of phytoconstituent and followed by with biochemical parameters for establishment of valid scientific data.

Statistical Analysis

The data were analyzed statistically by a one-way analysis of variance followed by a multiple reference test by Dunnet. The mean values for each parameter were estimated ± SD. Significance rates were held at $P < 0.05$. A two factors four levels factorial design was employed to estimate the effect of temperature and time on calculated yield. The percentage yield was taken as a response for evaluation of the design. The analysis of the design at a significance level $P < 0.05$ showed the 4 × 2 factorial design was significant. Furthermore, percentage protection was correlated with the dose and body weight of animals using 2 × 2 full factorial designs with replicate. Four experiments were constructed by considered varying the dose and body weight using software JMP version 11. Each parameters were tested at two levels, that is, dose (low, 250 mg and high, 500 mg) and body weight (low, 25 ± 5 and high 35 ± 5).

RESULTS

Yield of the Extract

The process of extraction is a multivariate process. The key factors selected for the extraction process (Microwave extraction) was the effect of time and temperature which play a major role on yield of the extractable component [Table 1]. A two factors four levels factorial design was employed to estimate the effect on yield. The design yielded 16 experimental runs. The percentage yield was taken as a response for evaluation of the design. The analysis of the 4 × 2 factorial design was significant and showed significant effect on the yield of the component. The response surface graph [Figure 2] depicts the same. Result showed that during 15 min extraction at 60°C temperature in microwave extractor, the DN root aqueous extract yielded higher (36.20%) than other selected parameters.

Phytochemical Screening

The chemical tests of an aqueous extract of DN root were carried out and revealed the presence of alkaloids, flavonoids, glycoside, and phenols, which play an important role in therapeutic efficacy.

Acute Toxicity

The acute toxicity study of ARDN was carried out at two different dose levels, that is, 2000 mg/kg, as well as 5000 mg/kg body weight. Two mice were died at dose level of 5000 mg. Hence, LD_{50} was fixed at 5000 mg/kg body weight. Hence, that $1/20^{th}$ and $1/10^{th}$ of the LD_{50} cut off value, that is, 250 and 500 mg/kg body weight were selected for investigation of anticonvulsant activity.

Anticonvulsant Activity

Effect of ARDN on PTZ-induced convulsion

PTZ is a convulsion inducing drug which showed convulsion in all the mice at dose level of 80 mg/kg when injected *s.c.* The ARDN extracts have been contrasted with the control group. ARDN pretreated mice (dose of 500 mg/kg *p.o.*) showed substantial delay in the initiation of seizures (7.37 ± 0.21***), decreased the duration (1.04 ± 0.11***) of PTZ-induced seizures in mice significantly, and found no mortality. Diazepam (2 mg/kg), blocked clonic convulsion, and mortality in mice against seizures induced by PTZ [Table 2].

Effect of ARDN on INH-induced convulsion

INH (300 mg/kg, *s.c.*) in all the mice has developed tonic clonic seizures. The control group was contrasted with ARDN groups. A dose of 500 mg/kg *p.o.* of ARDN delayed seizure initiation (43.03 ± 0.11***, $P < 0.001$) and reduced

Table 1: Percentage yield of the extracts of DN

	Temperature (°C)	Time (min)	Amount of extract (w/w)	% yield
Aqueous extract	20	5	0.94	9.40
	40	10	1.68	16.80
	60	15	3.62	36.20
	80	20	2.14	21.40

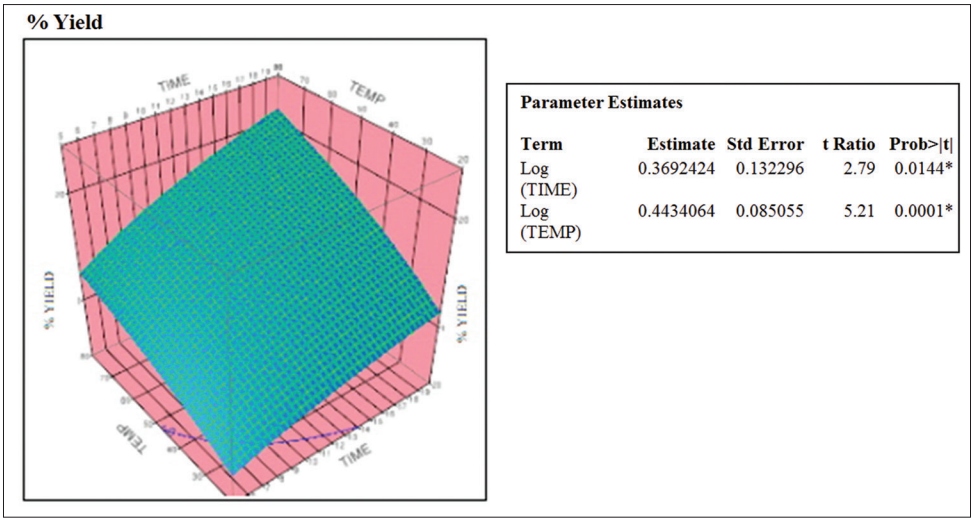

Figure 2: The response surface graph for yield with temp and time

duration of convulsion (1.12 ± 0.42***, $P < 0.001$) in mice against INH-induced seizure significantly. For ARDN (dose of 500 mg/kg *p.o*.), 75% of mice defended against INH-induced seizure. Diazepam (10 mg/kg *i.p.*), the standard anticonvulsant drug, totally abolished the convulsion in mice [Table 3].

Biochemical Estimation

Effect on brain lipid peroxidation

ARDN at dose level of 250 and 500 mg/kg *p.o*. showed significant dose-dependent inhibition in malondialdehyde (MDA) content (29.86%) in mice brain tissue as compared to control group [Table 4].

Effects on GSH level in mice brain tissue

Similarly, ARDN at dose level of 250 and 500 mg/kg *p.o*. showed significant dose-dependent increased in percentage content of GSH (58.22%) in brain of mice than the normal group [Table 4].

TLC Study

Various solvents were applied and standardized the method with mobile phase (toluene: ethyl acetate: formic acid, 7:5:1, v/v) for the presence of active constituents. The R_f found for Gallic acid and for Quercetin was 0.64 and 0.78, respectively, for ARDN extract [Figure 3].

HPTLC Study

The HPTLC fingerprinting analysis was studied according to the aforesaid procedure and the bands were observed on the HPTLC plates by compared with standard Gallic acid and Quercetin when scanned at wavelength of 366 nm and quantification was done by calculated the R_f values [Table 5]. The results from HPTLC finger print for ARDN sample (microwave extraction) revealed for the presence of Gallic acid and Quercetin in the extract [Figure 4a-c] were directly proportional with the percentage yield.

Table 2: Effect of ARDN on the Pentylenetetrazole-induced convulsion in mice

Groups	Dose mg/kg b.w.	Onset of clonic convulsion (min.)	Duration of convulsion (min)	Mortality/ used (%)
Control (Saline water) (*p.o*)	2 ml	1.68±1.28	3.18±1.16	5/8 (62.5%)
Standard (Diazepam) (*i.p*)	5	0.0±0.00***	0.0±0.00***	0/8 (0%)
ARDN (*p.o*)	250	4.06±2.04***	2.74±0.42***	2/8 (25%)
ARDN (*p.o*)	500	7.37±0.21***	1.04±0.11***	0/8 (0%)

All values expressed as mean±SD; n=8 mice in each group, by one-way ANOVA followed by Dunnet's Multiple comparison test (compared with control group) *P<0.05, **P<0.01 and ***P<0.001=Significant

Table 3: Anticonvulsant effect of ARDN on the isoniazid-induced convulsion in mice

Groups	Dose mg/kg b.w.	Onset of clonic convulsion (min.)	Duration of convulsion (min)	Protection (%)
Control (Saline water) (*p.o*)	2 ml	24.04±2.31	7.12±0.63	0.00
Standard (Diazepam) (*i.p*)	10	0.0±0.00***	0.0±0.00***	100
ARDN (*p.o*)	250	36.28±1.10***	3.04±1.10***	37.50
ARDN (*p.o*)	500	43.03±0.11***	1.12±0.42***	75.00

All values expressed as mean±SD; n=8 mice in each group, by one-way ANOVA followed by Dunnet's multiple comparison test (compared with control group) *P<0.05, **P<0.01 and ***P<0.001

Table 4: Effect of ARDN on biochemical parameters

Groups	Dose mg/kg. B.w.	Lipid peroxidation *n* moles of MDA/ mg of protein	Decrease in MDA (%)	Glutathione micro moles/mg of protein	Increase in GSH (%)
Control (Saline water) + PTZ	2.0 ml	0.634±0.03	0.00	10.63±0.22	0.00
ARDN + PTZ	250	0.342±0.11***	33.21	15.74±0.11***	52.34
ARDN + PTZ	500	0.471±0.02***	29.86	17.30±0.20***	58.22

All values expressed as mean±SD; n=8 mice in each group, by one-way ANOVA followed by Dunnet's multiple comparison test (compared with control group) *P<0.05, **P<0.01 and ***P<0.001

Table 5: Quantification of quercetin and Gallic acid in ARDN extract

Solvent	% yield	Rf		Amount of constituents (mg/100 g)	
		Gallic acid	Quercetin	Gallic acid	Quercetin
Aqueous	36.20	0.64	0.78	0.049	0.086
	21.40	0.64	0.78	0.002	0.012

Figure 3: TLC of DN aqueous extract

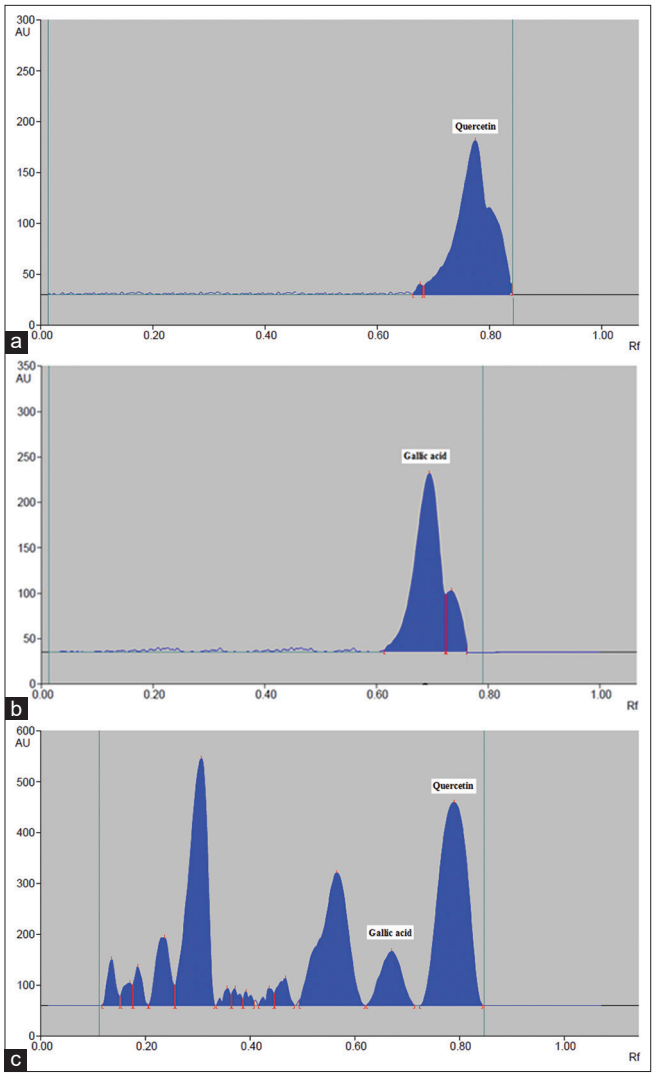

Figure 4: (a) HPTLC chromatogram of Standard Quercetin. (b) HPTLC chromatogram of standard Gallic acid. HPTLC chromatogram of aqueous extract of DN root. (c) HPTLC chromatogram of ARDN extract

Correlation Study

The percentage yield was correlated with amount of phytoconstituent which revealed positive linear correlation [Table 6]. Thereafter, yield was correlated biochemical parameters and result showed that % yield has impact on increased GSH content and reduction in lipid profile which was high statistical significant [Table 7]. Furthermore, body weight of animals and dose of the ARDN were correlated with overall percentage protection of anticonvulsant activity. The interesting positive significant result was observed when dose was correlated with overall percentage protection, but no significant result was observed with body weight of mice [Figure 5a-c; Table 8].

DISCUSSION

The percentage yield is the key predictor for any therapeutic efficacy. While in this present study, the yield calculation was done to reveal efficacy as an anticonvulsant action. The yield is, further, varied with the methods of extraction and extraction time and temperature, in which previous scientific literatures have already developed.[24-26] The same finding was found in the current investigation, where the extraction temperature and time play a major role in high yield. In the present analysis, the microwave extraction technique was used to get rapid extraction, using less solvent in less time. Some literatures disclosed the advantages of microwave extraction for plant drugs,[27-30] where less solvent volume was used to achieve high yield with less time span. Thereafter, aqueous solvent was selected in the study, because water is a strong polar solvent and it undergoes greater microwave absorption, which with its high dielectric constant efficiently transformed into heat. It is due to the interaction of microwave radiation with the solvent added and sample by conduction mechanism, where disruption of hydrogen bonding of water molecules occurs. This resulted in successful migration of dissolved ions and allows solvent to penetrate inside the cell sample which increases maximum yield.[31,32] Therefore, in present, investigation microwave extracted aqueous solvent resulted higher yield.

The qualitative determination of the phytoconstituents by means of different chemical identifications is important to confirm the existence of groups of constituents in plants ascertaining specific therapeutic effectiveness. In this present study, ARDN was chemically tested and the presence of several essential active compounds was revealed. The difference in the findings may have been due to the solvent impact and condition followed for the extraction. Perhaps, microwave extraction is

Table 6: Correlation study of % yield with amount of phytoconstituents

	% high yield	Content of Quercetin	Content of Gallic acid
% high yield	1		
Content of Quercetin	0.944*	1	
Content of Gallic acid	0.944*	1.00	1

Computed "r" for every pair of data set, confidence interval >95%.
*=Significant at $P<0.05$

Table 7: Correlation study of % yield with various biochemical parameters

	% yield	Decrease in MDA (ARDN 250 mg/kg B.w)	Decrease in MDA (ARDN 500 mg/kg B.w)	Increase in GSH (%) (ARDN 250 mg/kg B.w)	Increase in GSH (%) (ARDN 500 mg/kg B.w)
% yield	1				
Decrease in MDA (ARDN 250 mg/kg B.w)	0.400	1			
Decrease in MDA (ARDN 500 mg/kg B.w)	0.983***	0.529	1		
Increase in GSH (%) (ARDN 250 mg/kg B.w)	0.956**	0.359	0.904*	1	
Increase in GSH (%) (ARDN 500 mg/kg B.w)	1.00***	0.400	0.983***	0.956**	1

Computed "r" for every pair of data set, confidence interval>95%. ***=Significant at high level $P<0.001$; **=Significant $P<0.01$

Table 8: Analysis of variance and effect of tests on body weight and dose

Analysis of variance				
Source	**DF**	**Sum of squares**	**Mean square**	**F Ratio**
Model	2	0.46567271	0.232836	1040.851
Error	1	0.00022370	0.000224	Prob >F
C. Total	3	0.46589641		0.0219*

Effect Tests					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Body wt(25,35)	1	1	0.00573016	25.6156	0.1242
dose(250,500)	1	1	0.45994255	2056.087	0.0140*

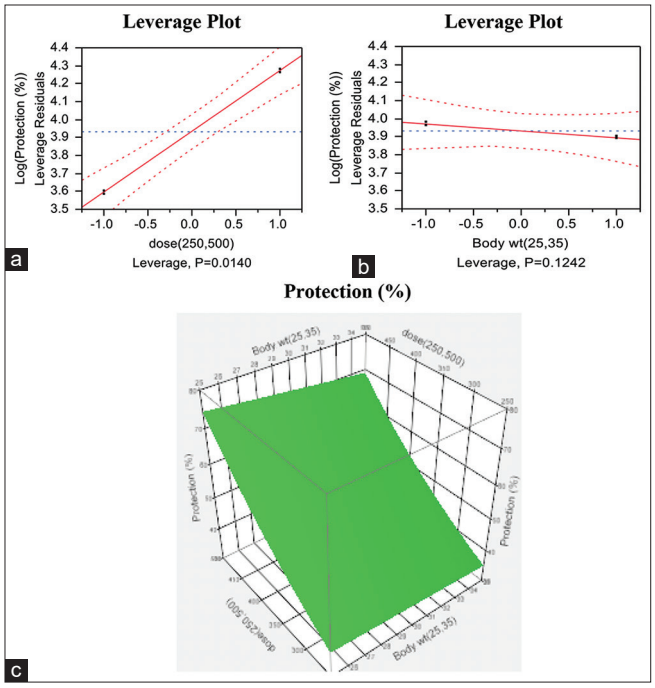

Figure 5: (a and b) Leverage plots for correlation study between % protection, dose, and body weight, respectively. (c) Factorial design of % protection

better for extraction of flavonoids and phenolic compounds using aqueous solvent.[33,34] The same trend was followed in the present investigation, where the presences of phenolics, alkaloids, and flavonoids were more prevalent in ARDN.

The study of acute toxicity study is an important parameter for knowing the protection of the specific plant extract. In the present research, the acute toxicity of ARDN was studied and resulted in a wide range of applications, that is, at 5000 mg/kg B.w, the maximum dose was tested which after oral administration was basically non-toxic and safe. The earlier literatures were also revealed the same which was similar with the reported data in the present study.[35,36]

Based on acute toxicity study, further anticonvulsant activity was evaluated on mice. Various animal models are developed to evaluate anticonvulsant activity. Based on that in the present experiment, PTZ and INH-induced models (involving gamma-amino butyric acid (GABAergic neurotransmission) were applied with diazepam as standard drug which was compared with the ARDN. Dose-dependent reduction of duration of convulsion was observed. This effect may be by interference with GABAergic neurotransmission. Furthermore, epilepsy is the main cause due to decreased function of $GABA_A$ synapses.[37] PTZ is an antagonist of GABA-A receptor which inhibits the GABA pathway to produces convulsion, mainly petit mal epilepsy. GABA is an important inhibitory neurotransmitter in the brain and acts on its own GABA receptors, whereas glutamate is an excitatory neurotransmitter, which acts through the N-methyl-D-aspartate (NMDA) and non-NMDA receptors. Activation of these receptors alters the actions of Na^+, K^+, Ca^{++}, and Cl^- ion channels which resulted excitement or inhibition of the neuron.[38] It was reported that the drugs that antagonize PTZ-induced convulsions are mostly effective in treatment of petit mal epilepsy. In this experiment, ARDN effectively acts against PTZ-induced convulsion, and hence, ARDN acts as effective drug for petit mal epilepsy by reduced action of T-type Ca^{2+} currents and also by enhanced $GABA_A$ receptor-mediated inhibitory neurotransmission. In addition, INH-induced mice model was performed as a potent monoamine oxidase (MAO) inhibitor and a glutamic acid decarboxylase inhibitor resulting

in increased brain monoamine content and simultaneous of GABA synthesis inhibition, respectively, and this mechanism impacts as an anticonvulsant effect[39] in this study.

In relation to the activity, plant constituents play a key role. Hence, in the present study, phytochemical screening through chemical tests was performed and many important constituents were identified. Among them flavonoids and phenolics were identified through TLC methods which are essential compounds in relation to anticonvulsant activity due to their antioxidant properties which enhance GABA levels in brain.[40] It was seen that the antioxidant defense mechanism decreases during epilepsy due to seizure action and therefore increases the amount of free radicals forms as a result induction of the oxidative stress occurred. Hence, flavonoids and phenolic compounds are more focus in the present study. Their antioxidant properties are due to presence of more phenolic groups in the structure which disrupts cellular oxidative processes in the central nervous system.[41] Recently, it was reported as an antidiabetic due to presence of flavonoid and quercetin.[42] It is also found that many flavonoids in the central nervous system may act as benzodiazepine-like molecules and modulate GABA-generated chloride currents in animal models of anxiety, sedation, and seizures.[43,44] Among them, quercetin works in very small doses and reduces convulsion by activating Ca^{2+}-activated K^+ channels and decreases extracellular K^+ leading to seizures improvement in hyperpolarization,[45] while Gallic acid elicits anticonvulsant activity through GABAergic pathway.[46] Furthermore, it was evident that greater value of GSH level, the lower the level of MDA level that means an inverse linear relationship between levels of MDA and GSH in the body helps in effective control of cunvulsant.[47] The same trend also observed with the present investigation. MDA is an important compound of lipid peroxidation and it is used as one of the indications of oxidative stress caused by free radicals,[48] because free radical injury is one of the important factors in development of seizure-induced neuronal damage.[49] In other hand, GSH is an important biomolecule that protects the cell against chemical-induced cytotoxicity by direct or enzymatic GSH-S-transferase conjugation with electrophilic compounds and reactive oxygen species, such as lipid hydroperoxides and hydrogen peroxide, and is affected by anticonvulsant treatments.[50] In the experiment, MDA level reduced and GSH level increased with the increased dose and followed the same trend as earlier literatures.[51] Furthermore, in the present analysis, the same finding resulted. HPTLC was subsequently performed to quantify these poly phenolic compounds for possible isolation of constituents for drug discovery. Finally, a correlation study was developed that provided a positive correlation with yield, content of phytoconstituents, and efficacy of the anticonvulsant activity.

CONCLUSION

The findings in this study showed that the ARDN had anticonvulsant activity which confirmed its successful application through through GABAergic receptor pathway with the presence of flavonoids and phenolics. The study subsequently verified that extraction method with standardized parameter increased the extract yield which provided a positive significant correlation for anticonvulsant activity. In addition, it was also identified that dose variability showed positive correlation with the protection of convulsion action but not significant with the body weight.

COMPETING INTERESTS

We have no competing interests.

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