Research Article
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Pharmacological evaluation of *Portulaca quadrifida*

Abstract

Herbal medicine usage increasing daily for various diseases. In some Indian villages, the villagers use only herbal medicine and get relief from diseases even they don't have complete knowledge of the herbal plants,[1–8] they just strictly follow the traditional system of herbal medicine.[9–12] In the searching such type of plant we found one interesting plant named *Portulaca quadrifida*[13,14] generally used for some sort of skin disorder by the local village people. Leaf extract contains alkaloids, glycosides, carbohydrates, starch, tannins, proteins, mucilage, and steroids. Leaf extract was pharmacologically evaluated for skeletal muscle relaxant activity, analgesic activity, wound healing activity, anti-anxiety activity, anti-convalescent activity, anti-depressant activity. Analgesic activity and wound healing activity showed better response.

Volume 5 Issue 6 - 2018

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Received: September 04, 2018 | **Published:** November 02, 2018

Materials and methods

Collection and authentication of plant

The leaves of '*Portulaca quadrifida*'[13,14] were collected in the month of March 2013 from the chilli fields of Mulkanoor, Karimnagar, AP, India. The plant was taxonomically identified by Dr. Musthafa, botanist, Kakatiya University, Warangal, AP, India. The leaves were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No#40 and stored in airtight container for future use.

Extraction

The coarse powder (1000g) was extracted with 1 litre of water by continuous hot percolation using soxhlet apparatus at 600c for 12hours. After completion of extraction, solvent was removed under reduced pressure. The dried extract was weighed and stored in desiccators.

Experimental animals

Albino mice and rats were produced from sainath labs uppal, Hyderabad and housed in institutional animal house. Further there were maintained as per CPCSEA guidelines.[15,16]

Acute toxicity testing

In many pharmacological screening, programmed acute toxicity on mice will be performed before going to other activities. In acute toxicity test, a single dose of the drug is used in each animal on one occasion only for determining gross behaviour and LD50.

In the present investigation, acute toxicity and gross behavioural studies were carried out in mice after administration of extract of *Portulaca quadrifida*.[17] Albino mice weighing 25-30g were selected, kept on overnight fasting, marked, and divided into 2 groups of six each. The two groups of mice received aqueous extract at the doses of 2000mg/kg and 5000mg/kg orally in the form of suspension. The mice were continuously and carefully observed for 2 hours, followed occasionally for 4 hours. The behavior (awareness, grooming, irritability, motor activity) and mortality of mice was observed up to 24 hours.[18,19]

Pharmacological investigation

Skeletal muscle relaxant activity

Muscle tone represents an important parameter in studying the physiology, pathology and rehabilitation. In different situations, like muscular dysfunction, normal daily activity or exercise, the muscle tone can be greatly influenced.

Grip strength method

This test is used to assess muscular strength in mice which can be influenced by a muscle relaxant or a sedative. Swiss albino mice of either sex weighing between 20-30gm are used. In a preliminary experiment the animals are tested for their normal grip strength by exposing them to horizontal thin metallic wire suspension about 30cm in the air, which they immediately grasp with their fore paws. The mouse is then released to hang on with its fore limbs. Normal animals are able to catch the wire with the hind limbs and climb on to it within 5sec. Animals, which fulfil this criterion are included in the test. After the oral or parentral administration of test or standard drugs the animals are tested every 15min for 2hrs. Animals which are not able to climb on to the wire with hind limbs within 5sec or fall off are considered to be impaired by drug effect.[20] After the completion of this test the animals are observed for their behaviour in the cage. If their behaviour and mobility in the cage appears to be normal, the disturbance of the grasping reflex can be considered to be caused by central relaxation. The animals were grouped as follows for the study.[21]

Wound healing activity

Male albino Swiss mice weighing 25-30g were used in wound healing model experiments.

Excision wound model

The dorsal skin of the mice was shaved. The mice were divided into three groups of six animals each. The animals were depilated on the paravertebral area prior to wound creation and predetermined area of 7mm×7mm skin in its full thickness was excised under ether anesthesia.[22] Control group were treated with plain base, standard will

receive the Povidone iodine 5% vice versa test group will receive the crude aqueous extract of *Portulaca quadrifida*.[23] The mice were allowed to recover from anesthesia and each mouse was then returned to his cage. Wounds were left undressed to the open environment and the animals were kept individually in separate cages and observed once in daily for 15 days.[24]

Measurement of wound area

The progressive changes in wound area were measured in mm at every 3 days interval. Progressive decrease in the wound size was monitored periodically.

Histological examination

On day 16 the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The tissue was processed in the routine way for histological evaluation. Five micrometer thick sections were stained with haematoxylin and eosin, the routine stain used in the histopathology, and recommended as a general survey stain. The tissue samples were evaluated for the following histological criteria; the extent of re-epithelisation, the maturation and organization of the epidermal squamous cells, the thickness of the granular cell layer, the degree of the tissue formation. The test and standard group results were compared with the control groups (Table 1) (Figure 1).

Table 1 Wound healing activity of aqueous extract of *Portulaca quadrifida*

S. No	Groups	Response (diameter in mm)				
		3days	6days	9days	12days	15days
1	Control	8.1±0.007	8.4±0.003	8.34±0.02	8.8±0.04	8.0±0.09
2	Povidone iodine ointment(5%w/w)	6.9±0.01**	6.9±0.02**	6.7±0.01**	6.6±0.02**	6.6±0.04
3	Extract (500g/Kg)	6.5±0.02**	5.9±0.01**	5.4±0.01**	5.0±0.03**	4.6±0.02

All values are expressed as mean±SD, n=6.*P<0.05;**P<0.01;***P<0.001

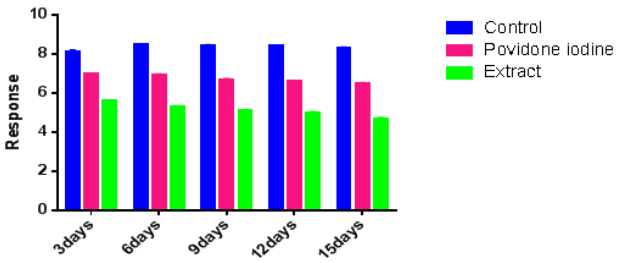

Figure 1 Wound healing activity extract of *portulaca quadrifida* of aqueous.

Analgesic activity

Acute pain is generally well accounted in terms of nociception, i.e. an excessive noxious stimulus giving risk to an intense and unpleasant sensation. On contrary most chronic pain states are associated with aberrations of the normal physiological pathway giving rise to hyper algesia. A variety of experimental pain models are available to demonstrate the nociceptive activity of drug and are used for routine screening of analgesics. Since different classes of analgesics vary in their mechanisms of pain relief, it is recommended not to rely on any one form of nociceptive test during the determination of analgesic efficacies. A great variety of nociceptive tests are currently used differ from each other by the nature of stimuli, parameters, and sites of application, nature of responses, quantization and apparatus.

Objectively, depending upon the nature of the stimulus, they can be classified into

a. Thermal stimulation methods (radiant heat/ hot plate).

b. Chemically induced nociception (acetic acid/ bradykinin/ formalin).

c. Mechanical stimulation method (compression at tip of tail).

d. Electrical stimulation method.

Hot plate method

The paws of rodents are highly sensitive to heat at temperature which do not damage their skin. They usually respond by jumping, withdrawing of paws and licking them. The time required for the onset of these responses in central animals is prolonged by centrally acting analgesics whereas peripheral analgesics and NSAIDs do not affect these responses.

Groups of six Swiss mice of either sex weighing between 20-25g are used for each dos. The commercially available Eddy's hot plate consists of an electrically heated surface. The temperature is controlled at 55-56°C. the animals are placed on the hot plate and the time until either licking of paw or jumping of animal occurs is recorded by a stop watch. The latency is recorded before and after 20, 60, 90min following s.c. or oral administration of the test compounds and the standard drug. The prolongation of latency times comparing values before and after the administration of drugs can be used for comparison using the student's t-test. ED50 values can be calculated using 3 doses of test standard producing dose dependent increase in latency. The method has been found suitable for screening centrally acting analgesics (Table 2) (Figure 2).

Table 2 Analgesic activity of aqueous extract of *portulaca quadrifida*

S. No	Groups	Response time (min)				
		30min	45min	60min	90min	120min
1	Control	7.4±1.74	7.4±1.85	7.2±1.6	8.8±0.4	8.0±1.41
2	Tramadol (30mg/Kg)	6.0±1.41	14.2±2.22***	22.2±2.13***	19.4±1.49**	8.2±0.74
3	Extract(500Mg/Kg)	6.6±1.3	15.4±3.0***	22.6±1.20***	18.4±2.15**	8.0±1.41

All values are expressed as mean±SD, n=6.*P<0.05;**P<0.01;***P<0.001

Citation: Vanga R, Srujana CY, Jyothi K. Pharmacological evaluation of *Portulaca quadrifida*. *MOJ Anat & Physiol.* 2018;5(6):335–341. DOI: 10.15406/mojap.2018.05.00221

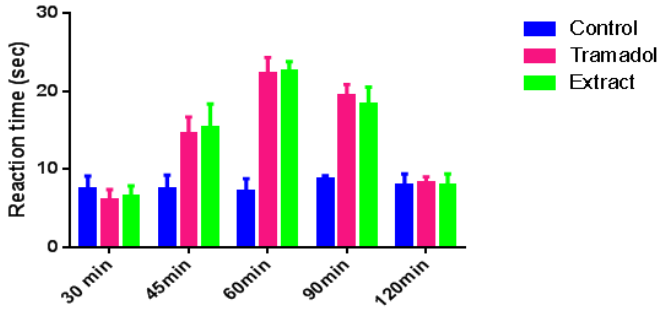

Figure 2 Analgesic activity extract of *portulaca quadrifida* of aqueous.

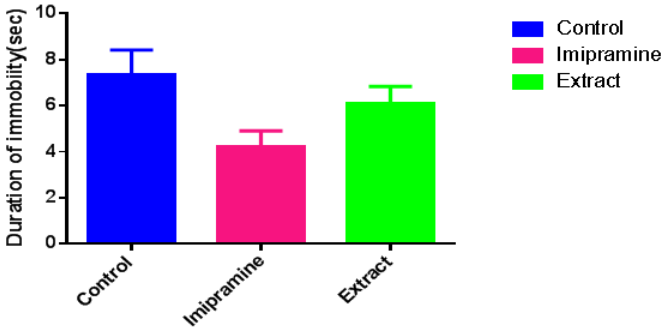

Figure 3 Anti-depressant activity of aqueous extract of *Portulaca quadrifida*.

Antidepressant activity

Depression is considered as an affective disorder characterized primarily by change of mood. It is associated with significant morbidity and mortality. Behavioural despair is a standard proposed model to test for antidepressant activity. It is suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can be reduced by agents which are therapeutically effective in human depression.

Forced swim test (FST)

Swiss albino mice weighing between 25-30g of either sex were randomly selected and animals are divided into 3 groups of 6 mice each.

Group 1 – Control group treated with 1% gum acacia (0.1ml/10gm).

Group 2 – Standard group treated with 25mg/kg of imipramine.

Group 3 – Test group treated with 500mg/kg of *Portulaca quadrifida*[25] aqueous extract.

The study was conducted for 15 days. Animals were administered drug orally every day for 15 days. Mice weighing 25-30g of either sex were used. 30min after the oral drug administration the experiment was carried out. Mice were individually forced to swim inside a vertical cylinder (height: 25cm, diameter: 12cm, containing 15cm of water maintained at room temperature). Mice when placed in the cylinder for the first time were initially highly active, vigorously swam in circles, trying to climb the wall or diving to the bottom. After 2-3 minutes, activity began to subside and there were phases of immobility or floating of increasing length. After 5-6 minutes, immobility reached a plateau where the mice remain immobile for 80% of the time. A mouse is considered immobile when floating motionless or making only those movements necessary to keep its head above water surface. Time of immobility is recorded in seconds every minute from 3 minute to 6 minutes.[17] After 6min mice were taken out, dried with a towel or tissue paper. The water was changed after each test because urine and the other chemicals released by the first mouse would affect the swimming pattern of the next mouse (Table 3) (Figure 3).

Table 3 Anti-depressant activity of aqueous extract of *Portulaca quadrifida*

S. No	Groups	Duration of immobility
1	Control	7.33±1.09
2	Imipramine(25mg/kg)	4.23±0.68***
3	Extract(500mg/Kg)	6.1±0.74***

All values are expressed as mean±SD, n=6.*P<0.05;**P<0.01;***P<0.001

Anti-anxiety activity

Anxiety is an emotional state caused by the perception of real or perceived danger that threatens the security of an individual. Anxiety disorders are considered the most common mental illness present in 15-20% of medical clinic patients.[26] Drugs like benzodiazepines, buspirone and propranolol are often used as first line approach in the management of anxiety related disorders.[27] These medications have many undesirable side effects and a significant number of patients are resistant to these drugs.[25] Hence there is a need for robust anxiolytic compounds that have lesser side effects. Anxiety a normal response to stress is a feeling of apprehension or fear, combined with symptoms of increased sympathetic activity. A clinical problem may arise if anxiety becomes severe or persistent, that interferes with everyday performance. Clinical subtypes on anxiety include panic disorder, agoraphobia, other phobias and generalized anxiety.[28] The prevalence of such syndromes in the general population is about 10-20%, and there is high rate of co-morbidity with depressive disorders.[29] The overall male to female ratio is 2:1. Although, the maximum prevalence of generalized anxiety and agoraphobia–panic is 50-64 age groups; the age of onset of most of anxiety disorders is in the young and adulthood (twenties and thirties). Current pharmacotherapy of anxiety revolves around the use of synthetic molecules as well as drugs obtained from the natural origin. However the drugs in current use are associated with side effect such as drowsiness, impaired motor activity, anticholinergic activity, and abusive tendencies.

Elevated plus-maze method

Elevated plus maze is most simple apparatus to study anxiolytic response of almost all type of anti-anxiety agents. Exposure of animals to novel maze alley evokes an approach avoidance conflict which is stronger in open arm. Rodents have aversion for high and open space and prefer enclosed arm. When animals enter open arm, they freeze become immobile, defecate and show fear like movements. The plasma cortisol level is also reported to be increased, as a reflection of anxiety. Major advantage of this test procedure are-

a. it is simple and less time consuming

b. no prior training

c. it is predictable and reliable procedure for studying anxiety response as well as anti-anxiety action of drugs.

Male wistar rats weighing 150-170g were selected and divided into three groups containing four animals in each group; these animals were fasted for 10-12 hours prior to experiment.[30] The groups were treated as follows,

The animals were individually placed in the centre of maze head facing towards open arm and the following were noted in a 5min time period

Citation: Vanga R, Srujana CY, Jyothi K. Pharmacological evaluation of *Portulaca quadrifida*. *MOJ Anat & Physiol.* 2018;5(6):335–341.
DOI: 10.15406/mojap.2018.05.00221

a. number of entries in open and closed arm (An arm entry is defined as the entry of all four paws in the arm)

b. average time each animal spends in each arm (Average time=total duration in the arm/no of entries)

The treated groups were subjected to the test after 20minutes of giving the treatment.

Anti-convulsant activity

A mental or neurological disorder encompasses broad range of conditions that result in dysfunction of brain, spinal cord and nerves. In this modern era, epilepsy is the most frequent neurodegenerative disease. Epilepsy is a disorder that is being viewed as a symptom of disturbed electrical activity in the brain. It is a collection of many different types of seizures that vary widely in severity, cause, consequence, appearance and management. Epilepsy implies a periodic recurrence of seizures with or without convulsions. There are around 20 to 70 new cases of epilepsy per 100,000 people per year. There are many classes of anti-epileptics that are of clinical usefulness with good prognosis for controlling seizures in most patients. Despite this, many patients have seizures that are not adequately managed by the established antiepileptic drugs. Moreover, the high incidence of adverse effects from the use of established antiepileptic drugs is also a source of widespread concern in patients who use them chronically. There are many mechanisms by which seizures can develop in either normal or pathologic brains. Three common mechanisms include:

a. Diminition of inhibitory mechanism (especially synaptic inhibition due to GABA).

b. Enhancement of the excitatory synaptic mechanism (especially those mediated by NMDA).

c. Enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents).

Different forms of human epilepsy may be caused by any one or combination of the above said mechanisms5-6. Both *in vivo* and *in vitro* models are available for the evaluation of anti-epileptic activities of drugs. In the *in vivo* methods, animals are used for the demonstration of an injury by exogenous agents of epileptic seizure on the brain with its physiological significance. *In vitro* models are employed to elucidate specific aspects of the mechanisms of injury.[31] *In vivo* animal models have been categorized by external agents and chemical agents that initiate the epileptic seizures, for e.g., maximal electro shock (MES) induced epilepsy, pentylenetetrazol (PTZ) induced epilepsy, picrotoxin (PTX) induced epilepsy and also other chemical agents like isoniazid, biccuculine (BCL), strychnine (STZ), aminopyridine, kainic acid induced epilepsy, models also kindled rat seizures. Mechanical methods like epilepsy induced by focal lesion, and genetic animal models of epilepsy, audiogenic models of epilepsy are available methods to screen the antiepileptic activities of drugs. The alternative drug therapy for the management of this disease can be by the use of medicinal plants and their active principles having little or without side effects.

Maximal electroshock induced convulsions

Different types of epilepsies that is grandmal, petitmal or psychomotor type, can be studied in laboratory animals. The maximal electroshock induced convulsions in animals represents grand mal type of epilepsy. Similarly chemo convulsions due to pentylenetetrazole will produce clonic type of convulsions resemble petitmal type of convulsions in man. In maximal electroshock (MES) induced convulsions electroshock is applied by corneal electrodes and cortical excitation is produced through stimulation of optic nerve.

The MES convulsions are divided into five phases such as,

a. Tonic flexion

b. Tonic extensor

c. Clonic convulsions

d. Stupor

e. Recovery or death

Male wistar rats weighing 150-170g were selected and divided into three groups containing four animals in each group; these animals were fasted for 10-12 hours prior to experiment. The groups were treated as follows,

The animals were held and the ear electrodes were clipped to ear and 150mA for 0.2 sec current was applied. The different stages of convulsions were a) tonic flexion, b) tonic extensor, c) clonic convulsions, d) stupor, e) recovery or death was noted. The times spent by the animals in each phase of convulsions were noted.[14]

Results

In the present study the aqueous extract of *Portulaca quadrifida*[13,14] was evaluated for its phytochemical constituents and pharmacological activities like a, analgesic, wound healing, skeletal muscle relaxant, and anti-depressant, anti-convulsant, anti-anxiety. The dried leaves powder was subjected to soxhlet extraction with water and % yield (w/w) was found to be 19.68%. The colour of extract was dark green with characteristic odour.

Phytochemical investigations

The preliminary phytochemical investigation of aqueous extract revealed the presence of phytoconstituents alkaloids, glycosides, proteins, terpenoids, carbohydrates, and tannins.

Acute toxicity studies

The acute oral toxicity study was carried out as per the OECD423 guidelines on mice and none of the doses tested, produced any signs of toxicity or mortality. Hence, the extract was considered to be safe up to the dose levels of 5000mg/kg bodyweight. Therefore pharmacological studies were carried out at doses of 500mg/kg, by oral administration.

Skeletal muscle relaxant activity

The results of skeletal muscle relaxant activity by Grip strength method one shown in table . The skeletal muscle relaxant effect was found to be high in test group compared with control and the skeletal muscle relaxant effect is less in test group compared with the standard group.

Wound healing activity

A better healing pattern with complete wound closure was observed in mice treated within 15 days while it took about 25-30 days in control mice with aqueous extract and standard povidone iodine. There was a significant reduction in wound area from day three onwards in treated mice and also on later days the wound closure was much faster than when compared with control mice.

Analgesic activity

The analgesic activity of the aqueous extract of *Portulaca quadrifida*[32,33] is as shown in Table 2 and Figure 2.The analgesic activity reached peak effect at 1 hour in both standard and test group and there after decreased. Although standard drug was administered

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parentally, orally administered test extract showed a comparable effect confirming the traditional use of the plant as wound healer.

Anti-depressant activity

The effect of aqueous extract of *Portulaca quadrifida*[25] on the immobility period duration in rats in forced swim test is as shown in Table 3 and Figure 3. The duration of immobility period was significantly low for the groups treated with standard drug (Imipramine) and extract when compared to the control. The duration of immobility was significantly high for extract treated groups when compared with standard group indicating that although anti-depressant effect is there, it is less compared to standard drug

Antianxiety activity

The results of anti-anxiety effect in mice for the aqueous extract of *Portulaca quadrifida,*[27] there is a significant increase in the number of entries in open arm as well as the time spent then in the standard and extract groups indicating that the extract has anti-anxiety effect comparable to that of standard drug diazepam.

Anti-convulsant activity

The effect of aqueous extract of *Portulaca quadrifida*[31] on MES induced convulsions in rats has shown a significant reduction in convulsions when compared with control. All phases have shown a decreased duration indicating anti-convulsant effect which is comparable to the standard anti-convulsant drug phenytoin.

Discussion

Portulaca quadrifida[13,14] leaf extract was evaluated for the presence of some phytoconstituents and also for pharmacodynamic activities. Preliminary screening for phytoconstituents revealed the presence of alkaloids, glycosides, carbohydrates, starch, proteins, terpinoids, carbohydrates, tannins, mucilage and steroids.

Skeletal muscle relaxant activity

The extract showed a significant skeletal muscle relaxant activity but when compared to the standard drug diazepam, activity lasted for only one hour and these results are similar to that reported in an earlier study[1] we conclude steroids, tannins are responsible for the wound healing activity. But at this stage, the phytochemical constituents responsible for this activity was not clean and through elaborated planned studies are required to say anything conclusively.

Wound healing activity

There was a significant improvement seen in wound healing in the test group, and the activity was more than that of the standard povidone iodine treatment. Since tannins are generally said to be possible for a faster wound closure,[24,34] we may say at this point that the tannins and steroids components of the extract contributed to a large extent in this activity. But more detailed study is yet to be performed for the confirmation.

Analgesic activity

The analgesic activity of the plant extract may be attributed to the presence of glycosides, alkaloids and other bioactive compounds as reported by[32,33] further studies are required to formulate a analgesic preparation from this extract and if further isolation of phytoconstituents from this plant is done, it may lead to valuable herbal analgesic products.

Anti-depressant activity

The anti-depressant activity of the plant extract, at a dose of 500mg/kg is significant when compared to control but much less than the standard. As reported in an earlier study[13,35] alkaloids, glycosides and proteins present in the plant may be responsible for this activity. But detailed study yet to be performed.

Anti-anxiety activity

The Elevated plus maze test is based on a premise where the exposure to an EPM evoked an approach–voidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in the open arm. Based on the herbal literature alkaloids, glycosides and may be responsible for the activity. More information needed for confirmation and detailed study is yet to be performed.

Anti-convulsant activity

A significant reduction in extensor, clonic convulsions and also reduction in stupor phase is seen in test group when compared to control group and almost all values are significant. As an earlier study performed by[13,35] reported on *Portulaca quadrifida* plant ethanolic extract alkaloids, glycosides, proteins and amino acids may be responsible for this activity. But at this stage, the phytochemical constituents responsible for this activity was not clear and through elaborated planned studies are required to say anything conclusively.

Conclusion

Based on the results of the present study, we conclude that the aqueous extract of *Portulaca quadrifida*[13,35] possess significant anti-convulsant activity, anti-anxiety activity, analgesic activity, wound healing activity, anti-depressant activity, skeletal muscle relaxant activity.[36–51] However, further studies are necessary to find the exact mechanism and to isolate the active compound(s) responsible for these pharmacological activities.[52–60]

Acknowledgements

None.

Conflict of interest

The authors declare there is no conflict of interest.

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Citation: Vanga R, Srujana CY, Jyothi K. Pharmacological evaluation of *Portulaca quadrifida*. *MOJ Anat & Physiol.* 2018;5(6):335–341.
DOI: 10.15406/mojap.2018.05.00221

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Citation: Vanga R, Srujana CY, Jyothi K. Pharmacological evaluation of *Portulaca quadrifida*. *MOJ Anat & Physiol.* 2018;5(6):335–341. DOI: 10.15406/mojap.2018.05.00221