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Assessment of Anxiolytic and Anti-depressant Activities of different Extracts of Catharanthusroseus Leaves in Experimental Animals

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Abstract--- *Catharanthus roseus leaves are investigated to assess anxiolytic and antidepressant activity in experimental animal models. Successively extracted pet. ether, chloroform and ethanolic extracts were used in the experiment. Anxiolytic and antidepressant activity was studied using elevated plus maze and forced swim test respectively. Oral administration of 200 and 400 mg/kg petroleum ether extract shows significant ($p<0.001$) and dose dependant anxiolytic and antidepressant activity. Diazepam 4 mg/kg, i.p. and Imipramine 15 mg/kg p.o. were used as standard drug. All the extracts at 100 mg/kg dose didn't show significant result in the experimental animals. In qualitative chemical test petroleum ether extract shows presence of tannins, flavonoids, glycoside, alkaloids and phenolic compounds. Petroleum ether extracts is rich in phenol content including flavonoids, which are reported responsive chemicals for anxiolytic and antidepressant activity in many preclinical studies. The assessed activities of Catharanthus roseus leaves could be due to polar and phenolic compounds.*

Keywords--- *Catharanthus Roseus, Anxiolytic, Antidepressant, Forced Swim Test, Elevated Maze.*

I. INTRODUCTION

Anxiety and depression are most dominant psychiatric disorder and expected to form the second common cause of morbidity by year 2020(Olatunji et al., 2007).Anxiety and depression has increasing significantly and gained much attention as the incidence of mental illnesses. Anxiety and depression may be due to multiple reasons.Asense of unease,feeling of worry, nervousness, or unease about something with an uncertain outcome is known as anxiety. Anxiety is a basic mental condition or can be a component of, or reaction to a primary medical disease(Kvaal et al., 2005). Anxiety disorders mostly involves the common behavioural and emotional problems which affects 1/8 th of the population worldwide, and have gained significant attention in research performed in the field of psychopharmacology(McNaughton and Gray, 2000). Different types of antianxiety drugs like benzodiazepines used to relieve the symptom of anxiety but due to their severe side effects none of these have achieved a successful and satisfactory response(Guy Edwards, 1981; Kuhn et al., 2010).

Depression affects on mood of person, feelings, health and performance(Yohannes et al., 2010). The depression includes biological and emotional symptoms. The retardation of thought, action and appetite are Biological symptoms. Whereas Emotional signs of depression consist of withdrawal from society, constant irritability and sadness,laziness, feeling of guilt, inadequacy and ugliness, indecisiveness and loss of motivation. Globally, more

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than 350 million population is affected by depression(World Health Organization, 2012).Depression itself indicates impairment of mood. Certain symptoms of disorders of depression overlie with the anxiety symptoms together with phobias and panic agoraphobia and obsessive-compulsive disorder. Depression is a global public health issue which involves substantial disability. Though a number of antipsychotic and antianxiety drugs like fluoxetine, imipramine,diazepam, alprozolamare available in the market to treat neurological disorders, but due to severe adverse effects and withdrawal symptom they are used with some restrictions and there are very few drugs which are used as anxiolytics and antidepressants (An and Lu, 2016; Chen et al., 2015). The motto of above investigation was to search a herbal product which is used for anxiety and depression with lesser side effects.

Catharanthus roseus (periwrinkle or vincarosea) is a well-known plant for anticancer activity.*Catharanthus roseus* is herbaceous plant 30-80 centimeter in height. The leaves are2.5- 9.0 centimeter long and 1- 3.5 cm in width, oval to oblong, glossygreen hairless ("The scope and practice of pharmacognosy," 2009).The leaves are in opposite pairs. Two types of flowers either entirely white, pink to violet or white coloured corolla with red eye with five petal like lobes.

Flowers are normally 2 to 3in cymose axillary clusters,bractate,pedicellate and complete, hermaphrodite. The fruit are nearly two to four centimeter in length and 3 milimeter in width having many black seeds.From vinca about 90 different types of alkaloids have been isolated. The leaves contain 150 useful alkaloids. Majority of them are the indole and dihydroindole derivatives(Heijden et al., 2004). The dimeric alkaloids, vinblastine and vincristine which are essential cancer drugs are present in leaves, while ajmalicine and serpentine are present in roots of *Catharanthus roseus.*

Worldwide *Catharanthus roseus* is used from ancestor time in the treatment of various diseases such as in India the leaves juice are used after to bee sting/ wasp sting(Nammi et al., 2003; Nayak and Pinto Pereira, 2006). Young leaves decoction is used in treatment of stomach cramps and in diabetis in Philippines. Decoction of root is used for intestinal parasitism.

In the menorrhagia leaf infusion is used. The bitter and astringent leaves are used as emetic. The roots are used as purgative, hemostatic, depurative, vermifuge and toothache remedies in Madagascar. The *Catharanthus roseus* leaves juice is used in the treatment of upset stomach and dyspepsiain Mauritius. In west Indies and south Africa the plant is used for the treatment of diabetes(van de Venter et al., 2008). To treat sore throat, laryngitis and chest ailments the gargle of plant is used in America. In menorrhagia and rheumatism leaves are used in Africa. Traditionally, the plant was used to relieve muscle pain and central nervous system depression (Rahman et al., 1984; Rajeswari, 2013)·The plant has various pharmacological effects such as anticancer, antidiabetic and antioxidant(Jayanthi et al., 2010),antimicrobial(Ghosh and Patil, 2010), antidiarroheal (Hassan et al., 2011), wound healing and antiulcer(Nayak and Pinto Pereira, 2006)etc. and the alkaloids obtained from the *Catharanthus roseus* plant possesses hypotensive, sedative, tranquilising and anti cancerous activities.

Present investigation was carried out to clarify conventional claim and the folkware use of *Catharanthus roseus* leaves.

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II. MATERIALS AND METHODS

Plant Material

Catharanthus roseus Leaves was obtained from Sangamner, Maharashtra, India. The identification of leaves was done by Dr.S. Jayanthi, Joint Director, Botanical Survey of India, Pune with a voucher specimen **(MTS 01**) and the specimen has been kept in herbarium of botanical survey of India, Pune.

Preparation of extracts:

Catharanthus roseus leaves was assembled and dried under shade. The dried leaves were powdered using mechanical grinder in to coarse form. Then coarse powder sieved through sieve no. 45(Mahapatra and Nguyen, 2007). Accurately weighed 500 gm of powdered material was packed in the soxhlet apparatus. Powdered material was successively extracted using the solvents petroleum ether, chloroform and ethanol with increasing polarity(Huie, 2002).

Preliminary Phytochemical Evaluation

For qualitative identification of phytoconstituents present in petroleum ether, chloroform and ethanolic extracts of leaves, the preliminary phytochemical screening(Kumar et al., 2013)was carried out.

Selection of Animals

Healthy male wistar rats weighing 200-250gm of 10-12 weeks and albino mice of both the sex weighing 25-30gm, were used for study. The animals were accommodate individually under standard condition of temperature (25± 1ºC), 12 hr light/dark cycle and fed with standard pellet diet and water *ad libitium*. The approval was obtained by institutional animal ethical committee. The approved protocol number of experiment as per CPCSEA guidelines is (approval no. MES/COP/IAEC/13/2015-16).

Acute Toxicity Studies

To study acute oral toxicity Swiss albino mice (25-30 gm weight) of both the genders were used. Mortality and behavioural changes in the animals were monitored for 48 hrs as per the OECD guidelines(OECD, 2001; Rathi et al., 2006).

Drugs and Solvents

The Solvents such as petroleum ether, chloroform and ethanol were procured from Merck. Drugs Diazepam, Imipramine were used as standard to study antianxiety and antidepressant activity.

Pharmacological Screening

Anti-anxiety Activity

Elevated plus maze test was used to study Anti-anxiety activity (Bertoglio and Carobrez, 2002; Hogg, 1996). Male wistar rats split into eleven classes. Each class consist of six animals each. The animals were categorised as shown in table 2. In short, group I was treated as control receives distilled water 10 ml/kg, p.o. Group II animals was treated as standard receives diazepam 4 mg/kg i.p. The III, IV and V Group of animals received 100,200 and 400 mg/kg of petroleum ether extract of *Catharanthus roseus* leaves suspended in acacia (3 % aqueous

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solution).Animals in group VI, VII and VIII received 100,200 and 400 mg/kg of chloroform extract and IX, X and XI group of animals received 100,200 and 400 mg/kg of ethanolic extract of *Catharanthus roseus* leaves.

Elevated plus maze apparatus consist of2 open arms of 35x5cm and 2 closed arms of 30x5x15cm.The arms are extended from a central platform of 5x5cm.The floor and closed arms wall are made from wood. From the ground level the maze is elevated 50 cm above. The rats were kept in a pair for 10 days in the apparatus before the test. The investigator handles the rats on alternate days to reduce the stress. After 30 min and 60min of oral administration of the drug and extracts each rodent was placed in the centre of the maze facing towards the surrounded arms.

When the rat putall his 4 paws over the marking line of respective area it is considered as entry in that area. In the 5 min duration period, number of entries in the open arm and the time spent in the open arm were recorded.

Antidepressant Effect

Antidepressant activity of *Cathranthus roseus* leaves was done by using forced swim test (Can et al., 2012; Slattery and Cryan, 2012). The animals were grouped into eleven groups (n=6) as shown in table 3. The Group I is a control group and animals received a vehicle (distilled water 10 ml/kg, p.o.). The standard i.e. GroupII gets Imipramine 15mg/kg orally. The animals in group III, IV and V were given 100,200 and 400 mg/kg of petroleum ether concentrate, animals in group VI, VII and VIII were receive 100, 200 and 400 mg/kg of chloroform extract and Group IX, X and XI were given ethanolic extract of *Catharanthus roseus* leaves. The cylindrical container having a diameter of ten cm and twenty five cm height had filled by water at (25 ± 1°C) up to nineteen cm depth. The immobility of each rat was judged. The rat was consider as immovable when it ceased struggling and remain floating in the water. The duration of immobility during the six min test was scored.

III. RESULTS

Preliminary Phytochemical Evaluation

Various chemical tests were carried out in step with the literature cited in method. Group of chemicals were identified in chemical test as shown in table 1. Preliminary phytochemical tests of *Catharanthus roseus* leaves suggests presence of numerous phytoconstituents inclusive of alkaloids, terpenoids, flavonoids and phenols.

Table 1: Preliminary Phytochemical Evaluation of Catharanthusroseus Leaf Extract

Sr.No.	Phytochemical Constituents	Petroleum ether extract
1	Steroids	-
2	Saponins	-
3	Tannins	+
4	Alkoloids	+
5	Carbohydrates	-
6	Proteins	-
7	Amino acids	-
8	Flavonoids	+
9	Diterpenes	+
10	Phenols	+

The positive (+) sign indicates Presence and negative (-) indicates absence of group of phytochemicals.

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Table 2: Effect of Catharanthusroseus Leaf Extract on Anxiety by Using Elevated Plus Maze

Sr. No.	Groups	Treatment	No. of Entries in Open arm	Average time spent in open arm
1	Control	D/W10ml/kg, p.o.	4.50±0.42	7.66±0.42
2	Standard	Diazepam 4 mg/kg,i.p.	8.33±0.55**	15.66±0.66**
3	PEECR	100 mg/kg, p.o.	5.00±0.25ns	8.50±0.22ns
4	PEECR	200 mg/kg, p.o.	7.00±0.25**	9.90±0.22**
5	PEECR	400 mg/kg, p.o.	7.33±0.21**	10.50±0.22**
6	CECR	100 mg/kg, p.o.	4.66±0.21ns	8.16±0.16ns
7	CECR	200 mg/kg, p.o.	6.50±0.22ns	9.66±0.33*
8	CECR	400 mg/.o.kg, p.o.	6.83±0.30*	10.16±0.30*
9	EECR	100 mg/kg, p	5.16±0.40ns	8.83±0.30ns
10	EECR	200 mg/kg, p.o.	6.16±0.30ns	9.16±0.30ns
11	EECR	400 mg/kg, p.o.	6.33±0.21ns	9.73±0.21*

Values are Mean ± SEM, n=6,when compared with control by using one way ANOVA followed by Dunnette's multiple comparison test, ns- non significant,* P< 0.05, **P<0.01.

PEECR (petroleum ether extract), CECR (Chloroform extract) and EECR (ethanol extract).

Table 3: Effect of Catharanthusroseus Leaf Extract on Forced Swim Test

Sr. No.	Groups	Treatment	Duration of Immobility (Sec)
1.	Control	D/W10ml/kg, p.o.	222.17±1.35
2.	Standard	Imipramine 15 mg/kg,p.o.	123.50±2.12**
3.	PEECR	100 mg/kg, p.o.	171.33±1.05ns
4.	PEECR	200 mg/kg, p.o.	160.33±0.66**
5.	PEECR	400 mg/kg, p.o.	138.50±1.23**
6.	CECR	100 mg/kg, p.o.	181.00±1.34ns
7.	CECR	200 mg/kg, p.o.	164.33±0.71ns
8.	CECR	400 mg/kg, p.o.	145.67±1.43*
9.	EECR	100 mg/kg, p.o.	183.67±2.27ns
10.	EECR	200 mg/kg, p.o.	171.33±1.11ns
11.	EECR	400 mg/kg, p.o.	152.33±1.08*

Values are Mean± SEM, n=6,when compared with control by using one way ANOVA followed by Dunnette's multiple comparison test, ns- non significant,* P< 0.05, **P<0.01.

PEECR (petroleum ether extract), CECR (Chloroform extract) and EECR (ethanol extract) of *Catharanthusroseus* leaves were used for the activity.

Here, PEECR, CECR and EECR are petroleum ether, chloroform and ethanol extracts of *Catharanthusroseus* leaves.

Effect of Catharanthus Roseus Extracts on Anxiety

The **c**hronic use of *Catharanthus roseus* leaf extracts exerts an anxiolytic effect in rat. Diazepam prominently ($p<0.01$) increases the number of entries in open arms compared to control group. The petroleum ether extract of plant (PEECR) at dose of 400 mg/kg p.o. significantly increased ($p<0.05$) the number of entries and time spent in open arm when compared with control group (table2).At a dose of 200 mg/kg and 400 mg/kg the petroleum ether extract of *Catharanthus roseus* leaves shows dose dependent anxiolytic activity.

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Effect of Catharanthus Roseus on Forced Swim Test

The antidepressant effect of petroleum ether, chloroform and ethanol extracts of *C.roseus* leaves at the dose 100,200 and 400 mg/kg had been studing by observing the changes in the duration of immobility in the Forced swim test (FST) using Imipramine as standard drug. In FST, petroleum ether extract produced significant reduction ($p<0.01$) in the immobility period in comparison with that of control group in dose dependant manner at a dose of 200 mg/kg and 400 mg/kg body weight.

IV. DISCUSSION

Mental health was a much neglected field until recently. Depression, fear, uneasiness and insomnia are more wide spread comorbid mental conditions that have been treated with botanical medicines. Herbal medicines are more safe and present with lesser side effects as compared to standard psychopharmacological agents(Kessler et al., 2005). The anxiolytic and antidepressant impact of the petroleum ether, chloroform and ethanolic extracts of the *Catharanthus roseus* leaves were studied using different animal models of anxiety and depression. Anxiety is physiological as well as psychological behavioural response of a subject to real or particular threats. Elevated plus maze is broadly accepted model to assess the anxiolytic behaviour. In accelerated plus maze anxiety is because of worry of height. Diazepam is used as a standard anxiolytic and has been frequently employed as a reference compound to study anxiolytic property of drugs in rodents(Shafeen et al., 2012). A rise within the number of entries in open arm and increased time spent in open arm shows relieve of anxiety significantly ($p<0.005$). The petroleum ether extract shows significant anxiolytic effect when compared with control. 100 mg/kg portion of petroleum ether, chloroform and ethanolic concentrate of *Catharanthus roseus* leaves was found to be non significant.

The constrained swimming test is broadly utilised and acknowledged model to check antidepressant activity(Santosh et al., 2011). In the FST, rodents are compelled to swim in limited space from which they cannot escape. This actuates a condition of social depression in animals, which is claimed to professed to repeat a condition like human depression(George et al., 2012).The chemical investigation of petroleum ether extract of *Catharanthus roseus* leaves shows presence of some important chemicals class. The extract showed positive chemical test for alkaloids, tannins, flavonoids and other polar phenolic compounds. Flavonoids were identified as anxiolytic agents in recent literature(Negri et al., 2012).Apigenin and kaempferol derivatives present in the plant has been reported as anxiolytic agents. The aqueous removes of P. edulis and P. Alata induced anxiolytic movements in rodents without disturbing memory forms, and the distinctions in flavonoid substances were utilised to clarify the distinction saw in anxiolytic impacts of these plants(Barbosa et al., 2008). A few indole alkaloids have been utilised as antidepressants or give lead structures to its development(Hamid et al., 2017). Based on findings and traditional uses, plants having a store of indole alkaloids are important beginning stage for the improvement of future antidepressants. According to the literature it is recognized that, plants possessing polar chemicals like flavonoids, phenolics and alkaloids shows anxiolytic and antidepressant effects. Hence, from our results of chemical tests of bioactive petroleum ether extracts, we claim to possess the activity due to above constituents.

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V. CONCLUSION

The petroleum ether extract shows significant anxiolytic and antidepressant activity in dose dependant manner compared to chloroform and ethanol extract. The anxiolytic and antidepressant effects may be due to one more chemicals found in bioactive extract. Alkaloid, tannin, flavonoid and other polar phenolic compound present in the petroleum ether extracts of *Catharanthus roseus* leaves. Hence, more studies will be required for identification of the active chemical constituent which are responsible for the observed anti-anxiety and antidepressant effect.

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