Anti anxiety activity of *Citrus limetta* fruit extracts on the elevated Plus maze model in mice

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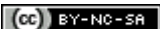
ABSTRACT

Citrus limetta, also known as, 'Sweet Lime' or 'Mosambi. Traditionally the plant has been used in the treatment of peptic ulcers, respiratory problems, digestion, cancer, jaundice and anxiety. However there have no pharmacological work done on Anxiolytic activity of *Citrus Limetta* fruit extracts. So, the present study have been designed to study the anxiolytic effects fruits extracts of Petrolium ether, Chloroform, Methanol and water using elevated plus maze model in swiss albino mice by different doses 100,200,400mg/kg. Diazapam 2mg/kg use as standard. Results displayed that methanol extract of fruits 400mg/kg dose *Citrus Limetta* increased the average time spent in the open arms of the EPM which was compared with the effect of diazepam. Hence this study shows that the plant has potentially antianxiety agent.

Key words: *Citrus limetta,* Petrolium ether, EPM, antianxiety agent

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INTRODUCTION

Anxiety is a characteristic human response that includes both personality and body [1]. Anxiety is characterized by a diffuse, undesirable, unclear feeling of misgiving. Usually attending via autonomic side effects, for example, sweat, palpitations, cerebral pain, and snugness in the chest [2]. In spite of the fact that, benzodiazepines have been known as a viable treatment of tension issue, they have a few unwanted symptoms. Consequently, further research is important to discover new anxiolytic medications with less unfavorable impacts [3-5].

Writing audit uncovered that the utilization of plants in the administration of ailments has been since time relic, and ceaselessly developed after some time as correlative medication since they were promptly and economically accessible medicinal services choices. Medications segregated from conventional plants may have conceivable restorative consequences for uneasiness. Research led to think study anti-anxiety compounds natural for alternative therapy [5].

Citrus limetta belong to genus citrus belonging to the large family Rutaceae, containing 130 genera in the seven subfamilies with many important fruit and essential oil producers [6]. Among the Citrus fruits of commerce oranges (sweet, mandarin and sour) are the most important as fresh fruit and they contribute to roughly 80 percent of the world's Citrus fruit production [7]. *Citrus Limetta* possesses various pharmacological activities such as Antiseptic, carminative, diuretic, eupeptic [8] and antimalerial [9], analgesic, antianaemic, antiemetic, antisclerotic, antipyretic, antiseptic, demulcent, moisturizing [8] kill intestinal worms [10], used for treating snake bites in North-Western Colombia [11] used to treat abundant menses [12] The literature review revealed that the plant fruits have been used for anxiety treatment but no biological data are available to support such facts. So, the present study have been designed to study the anxiolytic effects fruits extracts of Petroleum ether, Chloroform, Methanol and water using elevated plus maze model in swiss albino mice.

MATERIAL AND METHODS

Authentication: The fruits of *Citrus limetta* (family Rutaceae) were collected from a cultivated local nursery Amritsar. The herbarium was prepared by using collected plant material and Dr. K. Madhava Chetty (Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India) authenticated the plant under voucher specimen no.1241, dated May 28, 2016.

Animals: The experimental animals Swiss albino mice (20-25 gm) of either sex were procured from the central animal house, Animal House, Pinnacle Biomedical Research Institute (PBRI), Bhopal The animals were given standard laboratory feed and water ad libitum., both being withdrawn 12 hrs prior to experimentation. The experiments were performed between 8.00 to 12.00 hrs. The experiments were conducted in a semi sound proof laboratory. The biological studies were carried out as per the guidelines of institutional ethical committee.

IAEC Approval: All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (CPCSEA Reg. No. 1824/PO/ERe/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/PN-17029.

Preparation of leaf extracts: The petroleum ether, chloroform, methanol and aqueous extract of dried fruits(100gm) were prepared by successive Soxhlet extraction (Fig. 4.1). Solvent was distilled off, extracts were weighed and percentage was calculated in terms of dried weight of plant material.

Phytochemical screening: The extracts were tested for the presence or absence of alkaloids, saponins, flavonoids, carbohydrates, tannins and proteins [13-15].

Evaluation of anxiolytic activity
Treatments
a) Control: vehicle {simple syrup I P + tween 80 (5%)} 0.25 ml.
b) Standard drug: diazepam {2mg/kg orally}
c) Test extracts:Fruits extracts {petroleum ether, chloroform, methanol & aqueous extract at different doses i.e. 100, 200, 400 mg/kg suspended in vehicle}

Elevated Plus Maze Model: The elevated plus maze is a well established animal model for testing anxiolytic drugs [16-17]. It has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds by decreasing anxiety, increase the open arm exploration time, anxiogenic have the opposite effect. Plus maze apparatus consist of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm) with an open roof and is elevated to a height of 25cm. [18].

Procedure:
i) Weighing and numbering of the animals was done. Then they were divided into different groups, each consisting of 5 mice. One group was used as control (vehicle) and second group for standard

drug (diazepam) treatment and other test groups for different extracts.

ii) The animals were placed individually in the center of the maze, head facing towards open arm and the stop watch was started and following parameters were noted for 5 min.

  a. First preference of mice to open or enclosed arm.

  b. Number of entries in open and enclosed arms (an arm entry defined as the entry of four paws into the arm).

  c. Average time each animal spends in each arm (average time = total duration in the arm/ number of entries).

iii) Vehicle was administered to the control group. Diazepam was administered to the standard drug treatment group and extracts were administered to the various test groups. After 45 minutes animals were placed individually in the centre of the maze and all parameters as described under step 2 were noted.

Statistical Analysis: The anxiolytic activities of the extracts, Diazepam and control was analysed by ANOVA, the test groups were compared with standard/control by Tukey's Multiple Range Test. Difference were considered significant at p<0.05.

RESULTS

Petroleum ether extract: Treatment with petroleum ether extract at dose 100, 200, 400 mg/kg did not show any significant increase in the time spent by mice in open arms of EPM (Table 1) and (Fig. 1)

Chloroform extract: Treatment with Chloroform extract at dose 100, 200, 400 mg/kg did not show any significant increase in the time spent by mice in open arms of EPM (Table 2) and (Fig. 2).

Methanol Extract: Treatment with Methanol extract at the dose of 100mg/kg showed a significant increase in the time spent by mice in the open arms of EPM (Table 3) and (Figure 3).

Aqueous Extract: As the dose was increased, increase in the anti anxiety activity was found but treatment with aqueous ext. at dose 100,200,400 mg/kg did not show any significant increase in the time spent by mice in the open arms of EPM (Table 4) and (Figure 4).

Phytochemical screening: The results of phytochemical screening of fruits extracts as shown in Table 5

DISCUSSION AND CONCLUSION

The essential oils obtained from the genus Citrus are recommended for the treatment of anxiety in aromatherapy (Lehrner et al., 2000; Komiya et al., 2006). The present investigation was aimed at evaluating the anti-anxiety activity of various fruits extracts of *Citrus limetta* Well authenticated fruits of *Citrus limetta* were subjected to successive and exhaustive extraction with standard solvents in the increasing order of polarity with a view to segregate their constituents on the basis of polarity. Aqueous extract was observed to give higher yield of extractives.

]Anti-anxiety activity of various extracts and volatile oil was evaluated using a standard model of anxiety - "Elevated Plus Maze". This model can be used to evaluate both anxiolytic as well as anxiogenic effect. The model was chosen as it is very effective, cheap, simple, less time consuming, requires no preliminary training to mice and does not cause much discomfort to the animals while handling. The model is principally based upon the observations that exposure of animals to the elevated and open maze alley evokes an approach avoidance conflict which is manifested as an exploratory cum fear drive.

In elevated plus maze model various results shows that methanol extract at a dose of 100mg/kg shows significant antianxiety activity comparable to diazepam. The methanol extracts at a dose of 100mg/kg shows values 21.858 ± 1.487 as compared to diazepam 22.564 ± 1.519 in average time spent in the open arm as show in Table 3 and Figure 3 where as Petroleum ether extract, chloroform extracts, and aqueous extracts devoid of antianxiety activity because there values are very less as compared to diazepam as shown in Table 1 and Figure 1, Table 2 and Figure 2 and Table 4 and Figure 4. The phytochemical screening shows the presence of carbohydrates, saponins, flavonoids, phenolic compounds and tannins, phytosterols in the methanol extracts which is responsible for antianxiety activity. Further investigations are necessary for providing pharmacological products of *Citrus limetta* and better understanding of anxiolytic properties and neurobiological mechanisms of *Citrus limetta* extracts.

Table 1: Effect of Petroleum ether extract of fruits of *Citrus limetta* on time spent in open arms as measured on EPM in mice

S. No	Treatment	Dose (mg/kg)	n	Avg. time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Pet. ether	100	5	10.576 ± 0.828 *
4	Pet. ether	200	5	10.664 ± 0.788 *
5	Pet. ether	400	5	11.516 ± 1.389 *

n=no. of animals

The values are expressed as mean ±S.E.M.

*$p < 0.05$ as compared to Diazepam

Table 2: Effect of Chloroform extract of fruits of *Citrus limetta* on time spent in open arms as measured on EPM in mice

S. No	Treatment	Dose (mg/kg)	n	Avg. time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Chloroform extract	100	5	13.864 ± 1.629*
4	Chloroform extract	200	5	14.984 ± 1.556*
5	Chloroform extract	400	5	6.946 ± 0.651*

n=no. of animals

The values are expresed as mean ±S.E.M.

*$p < 0.05$ as compared to Diazepam.

Table 3: Effect of Methanol extract of fruits of *Citrus limetta* on time spent in open arms as measured on EPM in mice

S. No	Treatment	Dose (mg/kg)	n	Avg. time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Methanol extract	100	5	21.858 ± 1.487
4	Methanol extract	200	5	20.386 ± 0.968
5	Methanol extract	400	5	11.162 ± 0.831*

n=no. of animals

The values are expresed as mean ±S.E.M.

*$p < 0.05$ as compared to Diazepam

Table 4: Effect of Aqueous extract of fruits of *Citrus limetta* on time spent in open arms as measured on EPM in mice

| S. No	Treatment	Dose (mg/kg)	n	Avg time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Aqueous extract	100	5	9.910 ± 0.844 *
4	Aqueous extract	200	5	10.754 ± 0.656 *
5	Aqueous extract	400	5	13.406 ± 1.587 *

n=no. of animals

The values are expressed as mean ±S.E.M.

*$p < 0.05$ as compared to Diazepam

Table 5: Phytochemical screening of fruits extracts

Test	Petroleum Ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
Alkaloids	—	—	—	—
Carbohydrate	+	—	+	+
Saponins	—	—	+	+
Proteins and Amino acids	—	—	—	—
Triterpenoids	—	—	+	—
Phenolic compounds and Tannins	—	—	+	—
Phytosterols	—	—	+	—
Flavonoids	—	—	+	—
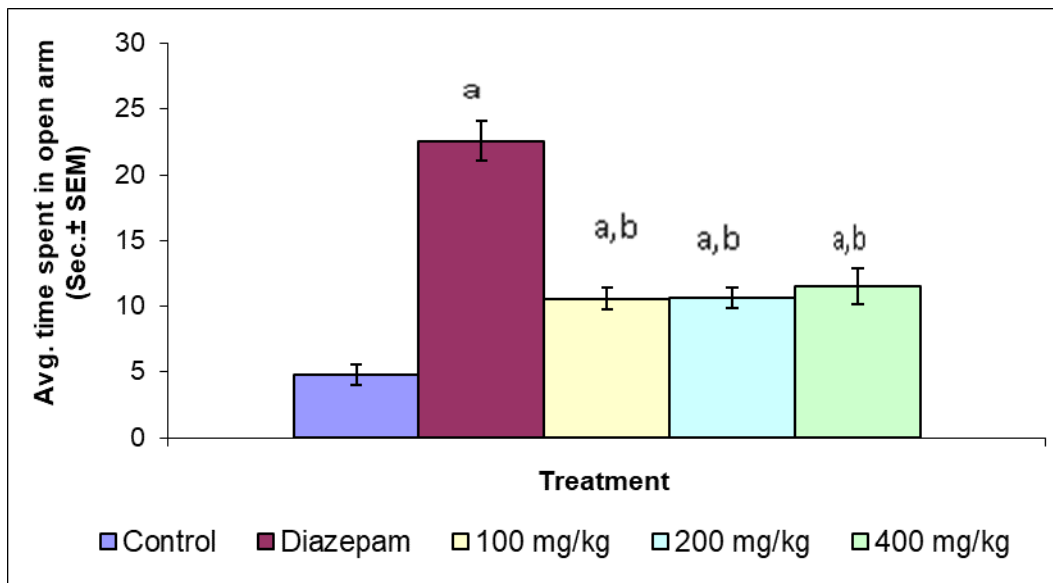
Figure 1: Effect of Petroleum ether extract of *Citrus limetta* on time spent in open arm
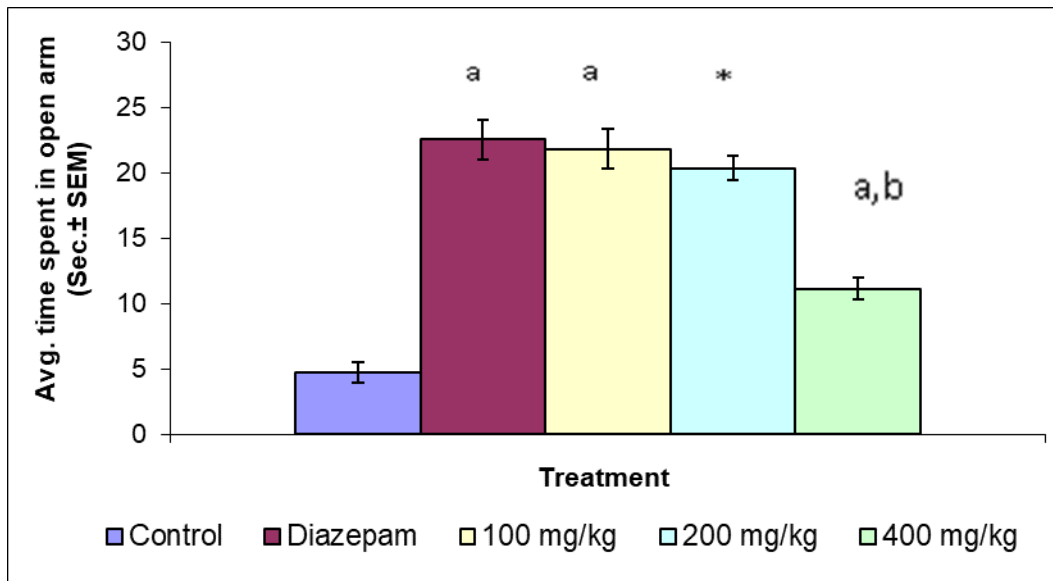The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.
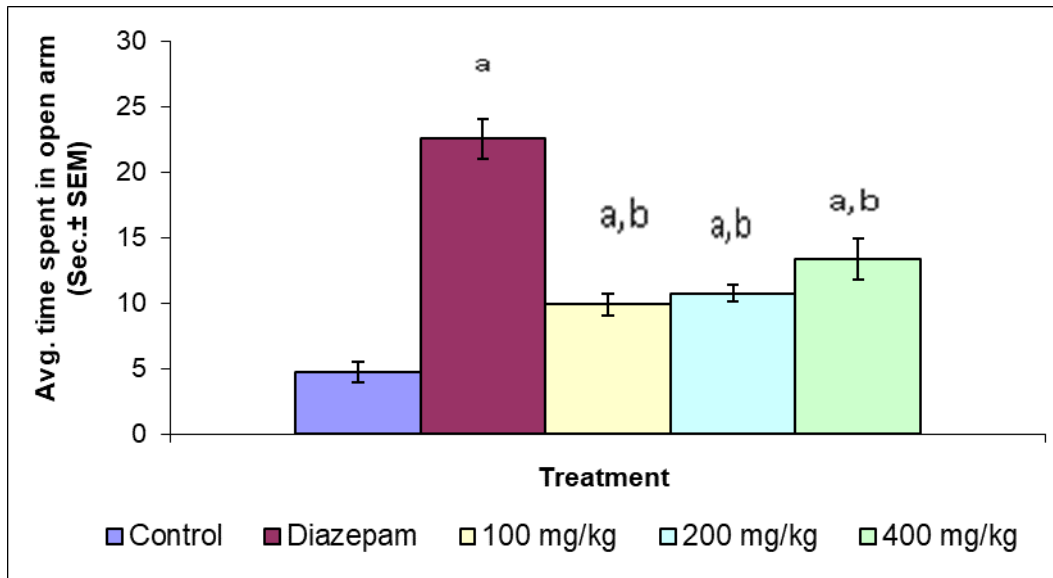a= p < 0.05 vs. Control (Vehicle); b= p < 0.05 vs. Diazepam (Standard drug)
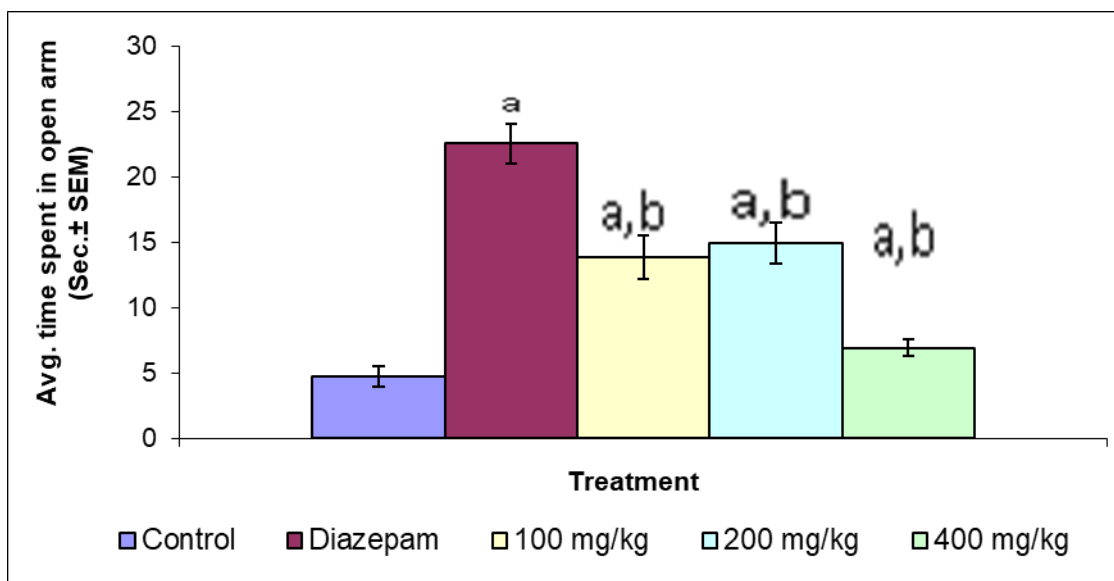
Figure 2: Effect of Chloroform extract of *Citrus limetta* on time spent in open arm
The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.
a= p < 0.05 vs. Control (Vehicle); b= p < 0.05 vs. Diazepam (Standard drug)

Figure 3: Effect of Methanol extract of *Citrus limetta* on time spent in open arm
The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.
a= p < 0.05 vs. Control (Vehicle); b= p < 0.05 vs. Diazepam (Standard drug)

Figure 4: Effect of Aqueous extract of *Citrus limetta* on time spent in open arm
The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.
a= p < 0.05 vs. Control (Vehicle); b= p < 0.05 vs. Diazepam (Standard drug)

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