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ANTI-ANXIETY ACTIVITY OF BIOACTIVE FRACTIONS OF LEAVES OF *CITRUS PARADISI* VAR. FOSTER AND MARSHSEEDLESS

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Abstract

Citrus fragrances have been attributed with mood enhancing properties by aroma therapists but not validated systemically. The ethyl acetate fraction (rich in flavonoids) of leaves of Citrus paradisi var. foster and marshseedless was subjected to bioactivity-guided fractionation to isolate chemical constituents responsible for anti-anxiety activity. The column chromatography of ethyl acetate fraction of Citrus paradisi var. foster and marshseedless yielded eight fractions (EAF_{1F}-EAF_{8F}) and twelve (EAF_{1M}-EAF_{12M}) respectively, which were screened for anti-anxiety activity employing elevated plus maze model. EAF_{2F} and EAF_{2M} of foster and marshseedless produced significant anti-anxiety activity at the dose of 10mg/kg p.o., upon acute administration in mice. EAF_{2F} and EAF_{2M} of foster and marshseedless was again subjected to column chromatography and yielded four sub fractions ($EAF_{2F.1}$-$EAF_{2F.4}$) for foster and five sub fractions ($EAF_{2M.1}$-$EAF_{2M.5}$) for marshseedless. $EAF_{2F.3}$ and $EAF_{2M.3}$ of foster and marshseedless at the dose of 10 mg/kg p.o. showed anxiolytic activity, which was statistically equivalent to the standard drug (Diazepam, 2mg/kg, p.o). $EAF_{2.3}$ of foster and marshseedless were again subjected to column chromatography and yielded four ($EAF_{2F.3.1}$-$EAF_{2F.3.4}$) and four ($EAF_{2M.3.1}$-$EAF_{2M.3.4}$) sub-fractions of foster and marshseedless and evaluated for anti-anxiety activity. $EAF_{2F.3.4}$ and $EAF_{2M.3.4}$ at the dose of 10 mg/kg p.o. showed anxiolytic activity. Preliminary phytochemical screening of $EAF_{2F.3.4}$ and $EAF_{2M.3.4}$ showed the presence of flavonoids. Further studies are in progress to find out the active isolated compounds from bioactive fractions.

Keywords: *Citrus paradisi; Foster; Marshseedless; Anti-anxiety activity; Diazepam; Elevated plus maze.*

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INTRODUCTION:

Stress and anxiety disorders are one of the most common mental disorders of modern world experienced by children and adolescents. In some circumstances, stress and anxiety are beneficial as they can motivate and help one be more productive, but when it becomes excessive, it impairs day to day life and leads to physical or psychological illnesses. Anxiety is a Central Nervous System disorder with emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat [1, 2]. Studies showed that lifetime prevalence rates of anxiety are between 13.6% and 28.8% in western countries and in 4.5% of the world population. It is more common in females than males. It has been reported that the prevalence of anxiety in adult males in India is 24.4% [3]. Anxiety disorders among higher secondary school students in India is 56.8%; among them 15% have panic disorder, 13% generalized anxiety disorder, 4% separation anxiety, 15.6% social anxiety and 9.2% school avoidance anxiety [4]. Conventional pharmacotherapy is associated with side effects such as psychomotor impairment, sexual dysfunction and dependence liability. Complementary and alternative plant-derived medications offer a fertile ground for investigating potential anxiolytic agents. A number of studies have been done on anti-anxiety activity of medicinal plants but major constraint towards development of a marker compound responsible for anti-anxiety activity and non availability of plant materials. This study entails to the development of marker compounds having anti-anxiety activity.

Citrus plants belonging to Rutaceae originated in Asia and now are cultivated all over the world. Citrus fruits are rich sources of bioactive compounds which show pharmacological activities such as antioxidant, antimicrobial, anti-tumor and anti-inflammatory activity [5], and thus citrus family is one of the most commercially important horticultural plants. The volatile oils obtained from genus *Citrus* (*Citrus paradisi*) have been recommended and used for the treatment of anxiety. A review of literature also reflects that *Citrus paradisi* is widely employed in herbal medicine and aromatherapy and significant work has already been reviewed and carried out by authors on the anxiolytic effects of the plant extracts [6-11]. In this study authors intend to isolate the bioactive fractions of *Citrus paradisi* var. foster and marshseedless leaves having anti-anxiety activity.

MATERIALS AND METHODS:

Plant material

The leaves of *Citrus paradisi* var. foster and marshseedless were procured and identified from a cultivated source of Punjab Agricultural University Regional Centre, Abohar (Punjab, India) in the month of March-April 2013. The plant material was dried in shade.

Animals

The experimental animals [Swiss albino mice (20-25 gm) of either sex] were procured from the Central Animal House, Akal College of Pharmacy and Technical Education, Sangrur. The animals were given standard laboratory feed and water *ad libitum*, both being withdrawn 12 hrs prior to experimentation. The experiments were performed between 6.00 am to 12.00 noon hrs. The experiments were conducted in a semi sound proof laboratory. The biological studies were carried out as per the guidelines of institutional animal ethical committee. The approval from the Institutional Animal Ethical Committee was taken before carrying out biological studies vide letter number ATRC/05/13 dated 04/05/13.

Preparation of various fractions and sub fractions

The active methanol extract of leaves of *Citrus paradisi* var. foster and marshseedless was suspended in water, placed in three-necked round bottom flask connected with Teflon stirrer and partitioned with ethyl acetate by heating (50^0C) for 30 min with continuous stirring. The procedure was repeated five times. All ethyl acetate fractions were pooled and solvent was recovered using Buchi 461 rotary vacuum evaporator. Thus, two fractions were obtained- ethyl acetate fraction (EAF) and the residual methanol fraction (RMF), and each fraction was evaluated for anxiolytic activity using EPM.

The bioactive ethyl acetate fraction (35g) of *Citrus paradisi var.* foster and marshseedless was loaded on a column packed with silica gel (#60-120), and eluted using Chloroform/Chloroform-Methanol/Methanol as the mobile phase. A total of 189, 187 fractions of *Citrus paradisi* var. foster and marshseedless respectively, 200ml each were collected. These were pooled based on similar thin layer chromatograms, to get 8 (EAF_{1F}-EAF_{8F}) and 12 (EAF_{1M}-EAF_{12M}) sub-fractions which were evaluated for antianxiety activity at various doses (5, 10, and 20 mg/kg p.o.) using Elevated plus model (EPM) apparatus. EAF_{2F} and EAF_{2M} of foster and marshseedless at the dose of 10mg/kg p.o. showed anxiolytic activity using EPM as comparable to diazepam. Bioactive fractions EAF_2 were subjected to column chromatography over silica gel (#230-400). Elution was done with petroleum ether, chloroform and chloroform-methanol and yielded four ($EAF_{2F.1}$-$EAF_{2F.4}$) and five ($EAF_{2M.1}$-$EAF_{2M.5}$) sub-fractions of foster and marshseedless respectively. $EAF_{2F.3}$, $EAF_{2M.3}$ of foster and marshseedless at the dose of 10 mg/kg p.o. showed anxiolytic activity using EPM as comparable

to diazepam. Further subfractuion $EAF_{2.3}$ of *Citrus paradisi var.* foster, marshseedless were again column chromatographed over silica gel (#230-400). Elution was done with petroleum ether, chloroform and chloroform-methanol and yielded four ($EAF_{2F.3.1}$-$EAF_{2F.3.4}$) and four ($EAF_{2M.3.1}$-$EAF_{2M.3.4}$) sub-fractions which were evaluated for anti-anxiety activity at various doses (2, 5, and 10 mg/kg p.o.) using EPM apparatus. $EAF_{2F.3.4}$ $EAF_{2M.3.4}$ of foster and marshseedless at the dose of 10 mg/kg p.o. showed anxiolytic activity using EPM as comparable to diazepam.

Statistics

The results are presented as mean ± Standard error mean (SEM). The anxiolytic activities of the fractions, diazepam and control were analyzed by using Analysis of variance (ANOVA) followed by Tukey's multiple range test/ Dunnett's test. Difference were considered significant at $p<0.05$.

RESULTS:

Citrus paradisi us traditionally used and medicinally important plant. Previous studies have shown significant anxiolytic activity of methanol extract of the plant. The fractionation of bioactive methanol extract of *Citrus paradisi* var. foster and marshseedless leaves yield two fractions i.e ethyl acetate fraction (EAF) and residual methanol fraction (RMF). EAF of *Citrus paradisi var.* foster and marshseedless at the dose of 20 mg/kg p.o. showed anxiolytic activity comparable to diazepam using EPM (Table 1).

Column chromatography of ethyl acetate fraction (EAF)

The bioactive ethyl acetate fraction (35g) of *Citrus paradisi var.* foster and marshseedless was loaded on a column packed with silica gel (#60-120), and eluted using Chloroform/Chloroform-Methanol/Methanol as the mobile phase. A total of 189 and 187 fractions of *Citrus paradisi var.* foster and marshseedless, 200ml each were collected. These were pooled based on similar thin layer chromatograms, to get 8(EAF_{1F}-$EAF_{8F)}$ and 12(EAF_{1M}-$EAF_{12M)}$ sub-fractions which were evaluated for antianxiety activity at various doses (5, 10, and 20 mg/kg p.o.) using EPM apparatus. EAF_{2F} and EAF_{2M} of foster and marshseedless at the dose of 10mg/kg p.o. showed anxiolytic activity using EPM as comparable to diazepam (Table 2).

Table 1: Anti-anxiety activity of EAF and RMF of using EPM

	Treatment	Average time spent in open arms(in sec)			
		Extracts		Control	
		EAF	RMF	Negative(Mean ± SEM)	Positive (Mean ± SEM)
***Citrus paradisi* var. foster**	Vehicle	-	-	7.987±0.613	-
	Diazepam	-	-	-	**24.567±0.683***
	20 mg/kg	**23.353±0.211***	18.743±0.219*	-	-
	40 mg/kg	**22.891±0.112***	18.871±0.157*	-	-
***Citrus paradisi* var. marshseedless**	Vehicle	-	-	10.023±0.850	-
	Diazepam	-	-	-	**26.133±0.692***
	20 mg/kg	**24.953±1.117***	18.321±0.193*	-	-
	40 mg/kg	**24.876±0.214***	17.343±0.331*	-	-

Values are Mean±SEM (n=6); One way ANOVA and Dunnett multiple range test. *p<0.05 compared to control.

Table 2: Antianxiety activity of various sub-fractions of ethyl acetate fraction (EAF) using EPM apparatus

	Treatment	Dose (mg/kg, p.o.)	Average time spent in open arms (in sec) (Mean ± SEM)
***Citrus paradisi* var. foster**	Control	Vehicle	7.987±0.613
	Diazepam	2	**24.567±0.683***
	EAF_{2F}	5	16.353±0.190*
		10	**22.878±0.644***
		20	**22.000±0.632***
***Citrus paradisi* var. marshseedless**	Control	Vehicle	10.023±0.850
	Diazepam	2	**26.133±0.692***
	EAF_{2M}	5	19.943±0.981*
		10	**26.023±0.131***
		20	**25.993±0.613***

Values are Mean±SEM (n=6); One way ANOVA and Dunnett multiple range test. *p<0.05 compared to control.

Bioactive fractions were subjected to column chromatography over silica gel (#230-400). Elution was done with petroleum ether, chloroform and chloroform-methanol. A total of 56 and 54 fractions (100ml each) of EAF2 of *Citrus paradisi var*. foster, marshseedless were collected respectively. These were pooled based on similar thin layer chromatograms, to get 4($EAF_{2F.1}$-$EAF_{2F.4}$) and 5($EAF_{2M.1}$-$EAF_{2M.5}$) sub-fractions which were evaluated for anti-anxiety activity at various doses (5, 10, and 20 mg/kg p.o.) using EPM apparatus. $EAF_{2F.3}$ and $EAF_{2M.3}$ of foster and marshseedless at the dose of 10 mg/kg p.o. showed anxiolytic activity using EPM as comparable to diazepam (Table 3).

Bioactive fractions $EAF_{2F.3}$ and $EAF_{2M.3}$ of foster and marshseedless were again column chromatographed over silica gel (#230-400). Elution was done with petroleum ether, chloroform and chloroform-methanol. A total of 95 and 84 fractions of $EAF_{2.3}$ of *Citrus paradisi var.* foster, marshseedless respectively, 100ml each were collected. These were pooled based on similar thin layer chromatograms, to get 4($EAF_{2F.3.1}$-$EAF_{2F.3.4}$) and 4($EAF_{2M.3.1}$-$EAF_{2M.3.4}$) sub-fractions which were evaluated for anti-anxiety activity at various doses (2, 5, and 10 mg/kg p.o.) using EPM apparatus. $EAF_{2F.3.4}$ and $EAF_{2M.3.4}$ of foster and marshseedless at the dose of 10 mg/kg p.o. showed anxiolytic activity using EPM as comparable to diazepam (Table 4). Preliminary phytochemical screening of $EAF_{2F.3.4}$ and $EAF_{2M.3.4}$ showed the presence of flavonoids.

Table 3: Antianxiety activity profile on EPM apparatus of various sub-fractions obtained after column chromatography of ethyl acetate fraction (EAF_2)

	Treatment	Dose (mg/kg, p.o.)	Average time spent in open arms (in sec) (Mean ± SEM)
***Citrus paradisi* var. foster**	Control	Vehicle	7.987±0.613
	Diazepam	2	**24.567±0.683***
	$EAF_{2F.3}$	5	20.446±0.864*
		10	**23.429±0.294***
		20	**23.120±0.473***
***Citrus paradisi* var. marshseedless**	Control	Vehicle	10.023±0.850
	Diazepam	2	**26.133±0.692***
	$EAF_{2M.3}$	5	23.764±0.787*
		10	**25.984±1.45***
		20	**25.047±1.69***

Values are Mean±SEM (n=6); One way ANOVA and Dunnett multiple range test. *p<0.05 compared to control.

Table 4: Anti-anxiety activity profile on EPM apparatus of various sub-fractions obtained after column chromatography of ethyl acetate fraction ($EAF_{2.3}$)

	Treatment	Dose (mg/kg, p.o.)	Average time spent in open arms (in sec) (Mean ± SEM)
***Citrus paradisi* var. foster**	Control	Vehicle	7.987±0.613
	Diazepam	2	**24.567±0.683***
	$EAF_{2F.3.4}$	2	18.400±0.102*
		5	20.441±0.478*
		10	**23.998±0.631***
***Citrus paradisi* var. marshseedless**	Control	Vehicle	10.023±0.850
	Diazepam	2	**26.133±0.692***
	$EAF_{2M.3.4}$	2	17.449±0.753*
		5	23.486±0.631*
		10	**25.745±0.431***

Values are Mean±SEM (n=6); One way ANOVA and Dunnett multiple range test. *p<0.05 compared to control.

DISCUSSION:

Anxiety disorders are serious medical illnesses that have affected 1/8th of total population worldwide irrespective of gender, age, religion, nationality and profession. A mental disorder has attracted the attention of researchers towards various pharmacotherapeutic approaches [12]. Benzodiazepines are used as a first line of treatment. Today, at least 20 million people worldwide are prescribed these "minor tranquilizers." Regular use of BZDs causes deterioration of cognitive functioning, addiction, physical dependence and tolerance [13]. Due to adverse effects associated with the synthetic drugs, researchers have been exploring natural resources based on traditional systems of medicine to come across safer and effective drugs [14]. The EPM is a novel, effective, cheap and simple method, requires no preliminary training of experimental animals, and does not cause much discomfort to them while handling. The fear due to height (acrophobia) induces anxiety, measured by time spent by mice in open arms of the EPM. Anxiolytic compounds, by decreasing anxiety, increasing the open arm exploration time; anxiogenic compounds have the opposite effect [15]. Mice constitute an invaluable tool for modeling human anxiety in its various forms as these display remarkable similarities on anatomical, physiological, biochemical, molecular and behavioral levels [16]. *Citrus paradisi* have a long history of use in alternative and traditional system of medicine for the treatment of mental disorders.

In the present study, bioactivity-directed fractionation of *Citrus paradisi var*. foster, marshseedless was carried out with a view to isolate the anxiolytic fraction. During the earlier studies carried out by authors, *Citrus paradisi* leaves were Soxhlet extracted successively with petroleum ether, chloroform, methanol and water. The four extracts were subjected to anxiolytic activity evaluation using EPM. It was observed that only the methanol extract exhibited anxiolytic activity at 100 mg/ kg, p.o. Thus, methanol extract of the plant was selected for bioactivity guided fractionation. Methanol extract when partitioned with ethyl acetate, yielded two fractions - EAF and RMF. EAF exhibited significant anxiolytic activity at a dose of 20 mg/kg, p.o. However, RMF did not show any anxiolytic effect on mice. Column chromatography of EAF yielded different sub-fractions and $EAF_{2F.3.4}$ and $EAF_{2M.3.4}$ of foster and marshseedless respectively showed anxiolytic activity at the dose of 10 mg/kg p.o. using elevated plus maze model of anxiety. Preliminary phytochemical screening of $EAF_{2F.3.4}$ and $EAF_{2M.3.4}$ showed the presence of flavonoids. Natural flavonoids like apigenin, 6-methyl apigenin, chrysin, hesperidin, luteolin, orientin, isoorientin and wogonin have been reported to possess anti-anxiety activity [17-21]. Thus, flavonoid constituents present in the plant may be responsible for its noted anxiolytic activity. Further studies are in progress to find out the active isolated compounds from bioactive fractions.

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

REFERENCES:

1. Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KR, et al. Mood disorders in the medically ill: scientific review and recommendations. Biol Psychiatry, 2005; 58: 175-189.
2. Gureje O, Von Korff M, Simon GE, Gater R. Persistent pain and wellbeing: a World Health Organization Study in Primary Care. JAMA, 1998; 280: 147-151.
3. Raakhee AS, Aparna N. A study on the prevalence of anxiety disorders among higher secondary students. GESJ: Education Science and Psychology, 2011; 1(18): 33-37.
4. Sahoo S, Khess CRJ. Prevalence of depression, anxiety, and stress among young male adults in India. J Nerv Ment Dis., 2010; 198: 901-904.
5. Mabberley DJ. Citrus (Rutaceae): a review of recent advances in etymology, systematics and medical applications. Blumea, 2004; 49: 481-498.
6. Gupta V, Bansal P, Kumar P, Shri R. Anxiolytic and antidepressant activities of different extracts from *Citrus paradisi* var. foster. J Pharmacy Res., 2009; 2: 1864-1866.
7. Gupta V, Bansal P, Kumar P, Shri R. Anxiolytic and antidepressant activities of different extracts from *Citrus paradisi* var. duncan. Asian J of Pharm and Clinical Res., 2010; 3: 98-100.
8. Gupta V, Bansal P, Kumar P, Kaur G. Pharmacopoeial standards and pharmacognostical studies of leaves of *Citrus paradisi* var. foster. Res J of Pharmacognosy and Phytochem., 2010; 2: 140-143.
9. Gupta V, Bansal P, Niazi J, Kaur G. Anti-anxiety Activity of *Citrus paradisi var.* star ruby Extracts. Int J of Pharm Tech Res., 2011; 2: 1655-1657.
10. Gupta V, Ghaiye P, Bansal P, Shri R. Pharmacopoeial standards and pharmacognostical studies of leaves of *Citrus paradisi* var. duncan. J of Pharmacy Res., 2011; 4: 1084-1086.
11. Gupta V, Bansal P, Kohli K, Ghaiye P. Development of Economic Herbal Based Drug

Substitute from *Citrus paradisi* (Grape fruit) for existing Anti-anxiety Drug Modules. Nat Prod Chem Res., 2014. http:// doi. org/10.4172/2329-6836.S1-001.
12. Bermudo-Soriano CR, Perez-Rodriguez MM, Vaquero-Lorenzo C, Baca-Garcia E. New perspectives in glutamate and anxiety. Pharmacol Biochem Behav., 2012; 100: 752-774.
13. Magnani P, Conforti A, Zanolin E, Marzotto M, Bellavite P. Dose effect study of *Gelsemium sempervirens* in high dilutions on anxiety-related responses in mice. Psychopharmacol., 2010; 210: 533-545.
14. Dhawan BN. 1995. Centrally acting agents from Indian plants. Rockville, MD: National Institute of Mental Health.
15. Vogel HG. 2002. Drug Discovery and Evaluation Pharmacological assays. 2nd ed. Germany: Springer-Verlag.
16. Hohoff C. Anxiety in mice and men: a comparison. J Neural. Transm., 2009; 116: 679-687.
17. Zanoli P, Avallone R, Baraldi M. Behavioral characterisation of the flavonoids apigenin and chrysin. Fitoterapia, 2000; 71: S117-S123.
18. Rocha FF, Lapa AJ, De Lima TCM. Evaluation of the anxiolytic like effects of Cecropia glazioui Sneth in mice. Pharmacol Biochem Behav., 2002; 71: 183-190.
19. Aguirre-Hernandez E, Gonzalez-Trujano ME, Terrazas T, Santoyo JH, Guevara-Fefer P. Anxiolytic and sedative-like effects of flavonoids from Tilia americana var. mexicana: GABAergic and serotonergic participation. Salud Mental, 2016; 39(1): 37-46.
20. Lotankar AR, Wankhede S, Sharma JB, Momin AJ. Anti-Stress Activity of Flavonoids Rutin and Quercetin Isolated from the Leaves of Ficus benghalensis. International Journal of Pharmacy and Pharmaceutical Research, 2016; 5(4): 5-19.
21. Gonzalez-Trujano ME, Hernandez-Sanchez LY, Ocotero VM, Dorazco-Gonzalez A, Fefer PG, Aguirre-Hernandez E. Pharmacological evaluation of the anxiolytic-like effects of *Lippia graveolens* and bioactive compounds. Pharmaceutical Biology, 2017; 55(1): 1569-1576.