Evaluation of Central Nervous System Acting Effects of Citrus Peel Essential Oils Extracted Using Enzyme Technology on Rodent Models

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Abstract-There is growing evidence of measurable effects of essential oils in animal brains and so more clinical research is required to validate their influence on the human central nervous system. This will enable us to discover essential oil-based drugs for treatment of mental illnesses such as depression, anxiety etc. Several methods have been developed to obtain oil from oil-rich plant materials using aqueous enzymatic methods. By using enzymes to mediate the extraction, it is possible to maintain mild conditions and effect superior extraction. The enzymes such as cellulase, hemicellulase, pectinase and protease are the most favourable enzymes for degrading the cell wall in oilseeds to loosen oil sacs embedded in the seed structures. Reduced equipment costs and energy consumption are also potentially possible, since oil and protein may be recovered simultaneously Therefore, the present study is proposed on quality and quantity enhancement of essential oils from citrus peel waste through enzymatic intervention and evaluates their effect on the central nervous system in Rodent Models. As an attempt to obtain the essential oil through hydro distillation HD and hydro distillation enzyme assisted HDEA was carried out. Several experiments have been conducted to determine the optimal process parameters for both methods, i.e., substrate to solvent ratio, extraction temperature, extraction time, enzyme loading, and incubation time etc. to Obtain essential oil from citrus peel collected from the local market of Dehradun. Total yield and physical characteristics like specific Gravity, viscosity, refractive index, acid, Saponification, iodine no etc. were compared. Both samples of oil CA-1 and CA-2 were further screened for their effect on Central Nervous System on the rodent model. Result indicated HDEA not only improved yield but also has sustainably stimulant effect on the central nervous system as compared to HD.

Keywords: Essential oil, Enzymes, Central nervons system.

Introduction

Essential oils are valuable plant products, generally of a complex composition comprising the volatile principles contained in the plant[1]. The oil droplets being stored in the oil glands or sacs can serve as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth, and stimulating healings. A vast variety of biological and pharmacological activities are associated with them. Experiments in animal models have exhibited the involvement of multiple neurotransmitter systems in the mode of action of essential oils, resulting in physiological effects in the brain. Also, the clinical trial study demonstrated the influence of essential oils in physiological parameters such as blood pressure, heart rate, respiratory rate, brain waves composition, and cortisol serum levels with concomitant psychological effects. Although there is growing evidence of measurable effects of essential oils in animal brains, more clinical research is required to validate their influence on the human central nervous system. This will enable the discovery of essential oil-based drugs for the treatment of mental illnesses such as depression, anxiety etc[2,3]. Generally, methods of extraction

followed for aroma and pigment from plant materials are solvent extraction, hydro-distillation, steam distillation, and supercritical carbon dioxide extraction. Several methods have been developed to obtain oil from oil-rich plant materials using aqueous enzymatic methods. Unlike traditional oil extraction methods, these new bioprocesses are performed without the use of organic solvents. By using enzymes to mediate the extraction, it is possible to maintain mild conditions and effect superior extraction. However, apart from laboratory trials, essential oil extraction using enzymes is largely an unexplored area. There is a great potential for this enzyme-based extraction technology with the selection of appropriate enzymes with optimized operating conditions. Various enzyme combinations are used to loosen the structural integrity of botanical material, thereby enhancing the extraction of the desired flavour and colour components. Recently enzymes have been used for the extraction of flavour and colour from plant materials, as a pre-treatment of the raw material before subjecting the plant material to hydrodistillation/solvent extraction. Deep knowledge of enzymes, their mode of action, conditions for optimum activity, and selection of the right type of enzymes are essential to use them effectively for extraction. The enzymes such as cellulase, hemicellulase, pectinase and protease are the most favourable enzymes for degrading the cell wall in oilseeds to loosen oil sacs embedded in the seed structures. Aqueous processing of oil-bearing materials eliminates the negative environmental impacts due to the emission of organic solvents and does not leave toxic or undesirable solvent residues in the resulting food products. Reduced equipment costs and energy consumption are also potentially possible, since oil and protein may be recovered simultaneously[4,5]. Therefore, the present study is proposed on quality and quantity enhancement of essential oils from citrus peel waste through enzymatic Intervention and evaluation of their effect on the central nervous system in Rodent models. Studies on these aspects are vital and would be very important in understanding enzymatic aided pretreatment role on essential oils yield and quality, this will also help in sustainable utilization of the waste.

Material and Methods

Preparation of substrate

Citrus peels were collected from a local market of Dehradun. Peels were cut into small slice and ground using a blender, followed by oven drying at 60°C for 48 hrs[6]. The ground dried peels were stored in an air-tight container at room temperature. The ground dried peels were stored in an air-tight container at ambient temperature.

Extraction of Essential oil

Hydro-distillation HD(CA-1)

A volume of 300 mL of distilled water was added into the 1-L round bottom flask containing 100 g of ground dried citrus peels. The round bottom flask was connected to the Clevenger-type apparatus. The temperature was set at 100 °C and the HD process was conducted for 3hrs[7]. Once the process was completed, the extract in the trap was collected and the yield was calculated and expressed as percentage in (w/w). The sample designated as CA-1 was stored in the amber airtight sealed vials at −20°C until required for further analysis.

Hydro-distillation with enzyme assisted HDEA (CA-2)

The fresh peels of citrus were cleaned, chopped, and dried and grounded. A total of 100 g of ground-dried citrus peels were mixed with 100 ml of 0.1 M acetate buffer, pH 5.0 in a 250-ml shake flask. A commercial cellulose solution at different enzyme loadings of 10-30

FPU/g-substrate was added into the solvent mixture. The mixture was incubated at 50 ± 2 °C, 200 rpm for 90 min[8]. The sample designated as CA-2 was filtered using a plastic sieve and stored in the amber airtight sealed vials at − 20 °C prior to hydrodistillation procedures.

Determination of yield

The extract in the trap was collected and the yield was calculated and expressed as a percentage (w/w)[9],calculated on moisture free basis. The oil was stored in the amber airtight sealed vials at 0 °C until required for further analysis. (Table -2).

Yield%w/w=Weight of essential oil (g)/Weight of substrate (g)×100%.

Physical Analysis

Essential oil along with aroma and flavours represent a highly complex class of natural products. Quantitative determination and physicochemical analysis of essential oils viz. refractive index, saponification value, acid value, ester value, optical rotation, specific rotation and specific gravity etc. were carried out by following standard methods of Guenther[10]. These methods have proved to be of great value in the essential oil industry because of their simplicity and reproducibility. The details are as being presented in Table -1.

Toxicity Study

Acute Toxicity Study: Five groups(n=5) of male albino mice were used in the acute toxicity study. Animal from all groups was fasted overnight and administered (P.O.) with a single dose (50, 100, 150, 250, 500, 1000, 1500, and 2000 mg/Kg body weight.) of samples CA-1 and CA-2 group of animals which received an equal volume of P.B.S. served as control. The study as performed in accordance with OCED guidelines[11]. Change in the behavior of animals were observed for 72 hours after extract administration For any sign of toxicity and mortality, animal were observed for 14 days.

CNS Activity

The locomotor activity (horizontal activity) can be easily measured using actophotometer which operates on photoelectric cells which are connected in a circuit with a counter. When a beam of light falling on the photocell is at on the animal a count is recorded. An actophotometer could have either a circular or square area in which the animal moves. Both rats and mice may be used for testing in this equipment.

Wistar rats of both sex (male and female) weighing 150-250 gms with a variation of ± 2.0 gm were taken for study. Test rats were kept in individual elastic cages with wire tops, prior to the use for screening. All the animals were fasted for at least 12 hours before use, allowing only access to water. The rats were divided into groups of five animals each. Each rat was weighed individually and was marked to distinguish one from another. The equipment was checked and it was ensured that all the photocell counter was recorded for each group for 10 mins. At the end of counting, each group of rats was removed from the counting chamber. Drugs were given orally as per the following schedule and after 60 minutes they were retested for activity scores for 10 minutes. The dose for drug was 100 mg/kg body weight of rat while the dose for standard reference drug i.e., gaba pantene was 250mg/kg body weight. Animals were divided into 5 groups and received different drugs as control group receiving Tween 80, Standard groups receiving Caffiene and Gaba Pantene and Test groups receiving CA-1 and CA-2.

CNS motor activity was calculated as per the following formula

$$\% \text{ CNS activity} = \frac{\text{Initial no. of counts - Final no. of counts}}{\text{Initial no. of counts}} \times 100$$

Statistically Data

All data were expressed as mean SEM + wherever applicable, the data were analysed statistically by student's t- test, using graph pad instant version 2.05a and one way ANOVA. The level of significance was $p < 0.05$ and n represents five per group.

Results and Discussion

As an attempt to obtain the essential oil through HD and HDEA, several experiments have been conducted to determine the optimal process Parameters for both methods, i.e. substrate to solvent ratio, extraction temperature, extraction time, enzyme loading, and incubation time Total yield of (w/w) and physical characteristics like specific gravity, viscosity, refractive index, acid, saponification, iodine no etc. were compared. Results are summarized in (Table-1). Both samples of oil CA-1 and CA-2 were further screened for their effect on Central Nervous System on rat model using actophotometer and acute toxicity studies. Results are summarized in Table-2.

Table -1 Yield and Physical Characteristics of Essential oils CA-1 and CA-2

Sample	Yield %w/w	Physical Characteristic	Specific gravity	Viscosity (cps)	Refractive index	Total acid number (mg KOH/g oil)	Iodine number (g/100g oil)	Saponification value (mg KOH/g oil)	Peroxide value (mEq O_2/kg oil)
CA-1	3.12±0.50 %	Clear solution with lemon like odour	0.692± 0.02	109±0.5	1.46	7	84	145	7.50
CA-2	5.02±0.50 %	Clear solution with lemon like odour	0.671± 0.01	117±0.45	1.45	5	89	132	7.01

Table -2 Effect of Essential Oil On CNS on Rodent Model

S.No.	Groups	Drugs	Dose	CNS Activity		% CNS Activity
				Loco motor count (Before)	Loco motor count (After)	
1.	B	Caffeine	100 mg/kg	37.6	63.2	68.08
2.	C	Gaba - Pantene	250 mg/kg	83	40.6	51.08
3.	D	CA -1	100 mg/kg Body weight	43	58.2	35.34
4.	E	CA -2	100 mg/kg Body weight	32.9	55.8	**69.60**

Gabba Pantene is CNS depressant and caffeine are CNS stimulant (count before is less and more after in stimulant while reverse in depressant)

As an attempt to obtain the essential oil through HD and HDEA, several experiments have been conducted to determine the optimal process parameters for both methods, i.e. substrate to solvent ratio, extraction temperature, extraction time, enzyme loading, and incubation time. HD with the process parameters of 1:3 (g/ml) of the substrate to solvent ratio, extraction temperature of 100 °C and 180 min of extraction time produced the highest amount of oil from citrus peels with a total yield of 3.12% (w/w) was obtained.

To extract the essential oil from the citrus peels, the HDEA method was conducted by adding cellulase to break down the cell wall of the peels to release out the essential oil. The cellulase loading was varied from 0 to 30 FPU/g and incubated for 60-150 mins. However, the results showed that adding the cellulase and prolonging the incubation time was not able to release out the essential oil. The enzyme loading of 10 FPU/g with an incubation time of 90 min was found to produce the highest yield. The cellulase helped in breaking down the cellular structures to obtain a greater permeability of the cell wall,

Physical characteristics like specific gravity, viscosity, refractive index, acid number, saponification value, iodine no etc were also compared. Results are summarized in (Table-1) Oil obtained by both techniques was found to be a clear solution with lemon-like odor with very little variation in other characteristics.

Both samples of oil CA-1 and CA-2 were further screened for acute toxicity studies and their effect on Central Nervous System on rat model using actophotometer. The results are summarized in (Table-2). Acute toxicity studies revealed that both samples CA-1 and CA-2 did not produce any toxic symptom and mortality when administered orally to mice at a dose of 50, 100, 150, 200, 500, 1000, 1500, 2000 mg/kg body weight in mice.

The aim of biological activity was to study effect of essential oil on the central nervous System. This was done in terms of locomotor activity of rats using actophotometer (activity cage).Most of the central nervous system acting drug influence, the locomotor activities in human beings and animal; the CNS depressant drug such as barbiturates and alcohol reduce motor activity while the stimulants such as caffeine and amphetamines increase the activity. In other words the locomotor activity can be in debt alertness of mental activity. Essential oils exhibit stimulation properties which lie in their structure closely in resemblance with actual hormones.[13,14] The penetration potential of these oils to reach the subcutaneous tissues is one of a key features of these oils used in aromatherapy. The mechanism of their action involves the integration of essential oils into a biological signal of the receptor cells in the nose when inhaled. The signal is transmitted to limbic and hypothalamus parts of the brain via olfactory bulb. These signals cause the brain to release neuro messengers like serotonin, endorphin etc., to link our nervous and other body systems assuring the desired change and to provide a feeling of relief. Serotonin, endorphin, and noradrenaline are released from calming oil, euphoric, and stimulating oil respectively to give expected effect on mind and body. From activity data summarized in Table-2. It is evident that in total both the oils HD and HDEA have exhibited CNS stimulant potential (35.34 and 69.60%) locomotor count respectively as compared to caffeine (68.08% locomotor count) but enzyme assisted extraction technique has sustainably enhanced the effect on central nervous system. Thus, HDEA technique tunes the quality and quantity of the essential oil from citrus peels.

Conclusion

Hydro distillation HD and Hydro distillation enzyme assisted HDEA had successfully produced the essential oil from citrus peels. The essential oil produced through both techniques shows comparable physical characteristics in terms of specific gravity, refractive index, acid number, saponification value. HDEE method

resulted in the rupture of essential oil glands, thus releasing the essential oil in good yield. Both samples of oil CA-1 and CA-2 were further screened for their effect on Central Nervous System on the rodent model. From the present study, it could be concluded that citrus peel which is a waste product can be utilized. The essential oil extracted from them using the HDEA technique not only increased the quantity of oil but also quality in terms of its CNS stimulant potential. A detailed study is required in this direction.

References

1. Bruneton, "Pharmacognosy, Phytochemistry, Medicinal Plants," England, Intercept, Hampshire, 1995, Pp. 434-436.

2. Lorena, R. and Lizarraga-Valderram Effects of essential oils on central nervous system: *Focus on mental health Phytotherapy Research.,* 2021, 35:657-679.

3. Ali, B.; Al-Wabel, N.A.; Shams, S.; Ahamad, A.; Khan, S.A. and Anwar, F. Essential oils used in aromatherapy: A systemic review. *Asian Pacific Journal of Tropical Biomedicine*, 2015,5:601-611. https://doi.org/10. 1016/j.apjtb.2015.05.007.

4. Rassem, H.H.; Nour, A.H. and Yunus, R.M. Techniques for extraction of essential oils from plants: a review. *Aust J Basic Appl Sci.,* 2016,10:117-127. [Google Scholar].

5. Hosni, K.; Hassen, I.; Chaâbane, H.; Jemli, M.; Dallal,i S.; Sebei, H. and Casabianca, H. Enzyme-assisted extraction of essential oils from thyme (Thymus capitatus L.) and rosemary (Rosmarinus officinalis L.): impact on yield, chemical composition and antimicrobial activity. *Ind Crops Prod.,* 2013,47:291-299.
doi: 10.1016/j.indcrop.2013.03.023.

6. Mohammad, Bagher.; Hassan, Pouraghdam.; Abbas, Hassani.; Lamia, Vojodi.; and Nader, Farsad-Akhtar Drying Method Affects Essential Oil Content and Composition of Basil (*Ocimum basilicum L*). *Journal of Essential Oil Bearing Plants,* 2010, 13(6):759 -766 ISSN Print: 0972-060X ISSN Online: 0976-5026.

7. Abdul-majeed, B.A.; Hassan, A.A. and Kurji, B.M. Extraction of oil from Eucalyptus camadulensis using water distillation method. *Iraqi J Chem Pet Eng.,* 2013,14(2):712.

8. Sowbhagya, H.B.; Purnima, K.T.; Florence, S.P.; Appu Rao, A.G. and Srinivas, P. Evaluation of enzyme-assisted extraction on quality of garlic volatile oil. *Food Chem.* 2009,113(4):1234-1238.
doi: 10.1016/j.foodchem.2008.08.0119

9. Furtado, F.B.; Borges, B.C. and Teixeira, T.L. Chemical composition and bioactivity of essential oil from blepharocalyx salicifolius. *Int J Mol Sci.* 2018,19(33):113.
doi: 10.3390/ijms19010033.

10. Guenther, E. The Essential Oil 1948,Vol 1 D. Van Nostrand Company, Inc publications.

11. AOAC Official Methods of Analysis. 14th Edition, Association of Official Analytical Chemists, Washington DC, 1985, No. 43.292. 7.001, 7.009, 7.006.

12. Oecd Series On Testing And Assessment Number 24 Guidance Document On Acute Oral Toxicity Testing. ENV/JM/MONO,2001,4.

13. Xiao Nan, Lv.; Zhu Jun, Liu.; Huan Jing, Zhang. and Chi Meng, Tzeng. Aromatherapy and the central nerve system (CNS): therapeutic mechanism and its associated genes, *Curr Drug Targets,* Jul 2013, 14(8):872-9.doi: 10.2174/1389450111314080007.

14. Perry, N. and Perry, E. Aromatherapy in the management of psychiatric disorders: clinical and neuropharmacological perspectives. *CNS Drugs*. 2006, 20(4):257-80. doi:10.2165/00023210-200620040-00001.PMID: 16599645.