THE ROLE OF BITTER ORANGE IN REDUCING FAT

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ABSTRACT
Obesity particularly central adiposity, has been increasingly cited as major health issue in recent decades. Indeed, some of the leading causes of preventable death and disability including heart disease, low back pain and specific types of cancer are obesity related. Sibutramine and orlistat are synthetic drugs for treating obesity for long-term use; they were launched several years ago. Natural products such as ephedrine, pseudo-ephedrine and caffeine were once extensively used in formulations for weight-loss, but they have side effects, which include death. In recent years, another natural product, synephrine, obtained from bitter orange which is extracted from traditional Chinese medicine Zhi Shi (or *Citrus Aurantium*), gains popularity in the market as a nutraceutical or dietary supplement for the regulation of appetite, body weight and athletic function. Extracts of *Citrus aurantium*, standardized for p-synephrine has been shown to increase energy expenditure, lipolysis and fat oxidation, activate brown adipose tissue by stimulating the systemic release of epinephrine and enhance weight loss in human. p-synephrine in combination with flavonoids and caffeine enhanced the weight loss.

KEYWORDS: Bitter orange, obesity, p-synephrine, weight loss, epinephrine.

INTRODUCTION
Bitter orange, also known as sour orange is a widely grown Citrus species. It is a small tree, about five meters tall, it is not widely used as an edible fruit due to its sour and bitter taste. Most of sour orange uses are medicinal rather than culinary. In today’s market, one of the primary interest constituent of Bitter orange peels is synephrine, due to its broad spectrum of medicinal and therapeutic activities including antifungal, antibacterial, antiviral activities and the most important is its recent use for weight reduction.

![Bitter orange](image)

Fig. 1 Bitter orange

Synephrine is an alkaloid derived from the peels of immature (green) fruits of the Seville orange botanically known as *Citrus aurantium*, of the family Rutaceae[1] and can exist as one of two enantiomers, d- and l-synephrine, that do not have identical pharmacological activities; however, literature survey revealed that synephrine is found as the l-isomer in *Citrus aurantium*, which is the more potent enantiomer.[2] The immature fruits are collected from May to June. It has subsequently been detected in *Evodia* and *Zanthoxylum* species and all plants of the family Rutaceae and trace levels (0.003%) in cactus species of the genera *Coryphantha* and *Dolichothele*. It also occurs in bacteria, invertebrates and vertebrates, including human in adrenal gland and probably in nerves of heart and brain although at extremely low concentration.[3]

History
The peel of the immature fruit is known as Chi shi or zhi shi in Traditional Chinese Medicine which was used to reduce food accumulation; to resolve phlegm and eliminate distention and fullness. The peel of the mature Bitter orange most likely originated in Southeast Asia and was initially propagated in India and Persia. In the 10th and 11th centuries Arab traders spread it around the Mediterranean countries: Syria, Palestine, North Africa, Sicily, Sardinia, the south coast of France and Spain. After the discovery of the New World it was introduced into the Caribbean. It is presently commercially cultivated in Southern Europe and other subtropical areas, particularly Spain, Portugal, Israel, and the various islands of the Caribbean.
Table 1: Synephrine Levels In Bitter Orange Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Mg</th>
<th>% (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruit</td>
<td>270 mg</td>
<td>0.020</td>
</tr>
<tr>
<td>Dried fruit</td>
<td>380 mg</td>
<td>0.352</td>
</tr>
<tr>
<td>Dried extract</td>
<td>3000 mg</td>
<td>3.003-3.079</td>
</tr>
</tbody>
</table>

Table 2: Synephrine Levels in Citrus Juices in mg/liter of juice.

<table>
<thead>
<tr>
<th>Fruit &amp; Variety</th>
<th>Synephrine level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td></td>
</tr>
<tr>
<td>Hamlin</td>
<td>22</td>
</tr>
<tr>
<td>Navel</td>
<td>15</td>
</tr>
<tr>
<td>Pineapple</td>
<td>27</td>
</tr>
<tr>
<td>Valencia</td>
<td>19</td>
</tr>
<tr>
<td>Tangerine</td>
<td></td>
</tr>
<tr>
<td>Dancy</td>
<td>125</td>
</tr>
<tr>
<td>Cleopatra mandarin</td>
<td>28</td>
</tr>
</tbody>
</table>

Chemical constituents
In addition to p-synephrine the bitter orange also contains the protoalkaloids tyramine, hordenine and N-methyltyramine.

Extraction of peels
100gm. of dried powdered fruit peels of *Citrus aurantium* were defatted with n-hexane for 24 hours and allowed to dry at room temperature. The defatted plant material was packed in a thimble and placed in soxhlet extractor. 500 ml of 80% methanol was used as a solvent and placed in a 1 liter round bottom flask fitted with a soxhlet extractor. The extraction was continued for 12 hours. The extract was filtered and concentrated under reduced pressure to dryness using rotary evaporator at a temperature not exceeding 40°C; then the dry extract was weighed and dissolved in 2N hydrochloric acid on a water bath, shaken and filtered; then the obtained filtrate was evaporated to dryness under vacuum to give white precipitate. Recrystallization was done to get pure compound.

Qualitative and quantitative estimation of synephrine by HPLC
HPLC was used for qualitative and quantitative estimation of synephrine. HPLC analysis was carried out . Identifications were made by comparison of retention times obtained at identical chromatographic conditions of analyzed samples and authentic standards. The mobile phase used was acetonitrile: water: trifluoroacetic acid (5:95:0.01) and the column used was C18 (150mm × 4.6mm/5um); flow rate was 0.6 ml/ min and detection by UV. Detector at λ 220 nm.[4]

Chemistry
In terms of molecular structure, synephrine has a phenethylamine skeleton, with a phenolic hydroxyl group, an alcoholic hydroxyl group and an N-methylated amino group. The amino group confers basic properties on the molecule, whereas the phenolic –OH group is weakly acidic. Common salts of racemic synephrine are its hydrochloride, oxalate and tartrate (sympatol).

The presence of the hydroxyl group on the benzylic carbon of the synephrine molecule creates a chiral center, so the compound exists in the form of two enantiomers, d- and l- synephrine, or as the racemic mixture, dl- synephrine.

Isolation of the active constituent
Isolation of synephrine was done by preparative layer chromatography, using glass plates coated with slurry of silica gel. The peels extract was applied as a concentrated solution in a row of spots using capillary tube. Reference standard of synephrine was applied at the right side of the baseline. The detection was done by using 2% ninhydrin in n-butanol spraying reagent. The bands corresponding to synephrine standard were scraped out and collected in a beaker and eluted with gentle heating and filtered; then the filtrate was evaporated to dryness under vacuum to give white precipitate. Recrystallization was done to get pure compound.

Identification of plant constituents by TLC
The extract was examined by TLC, using readymade plates of silica gel GF254 (20×20cm) of 0.25mm thickness. Detection was done by using 2% ninhydrin in n-butanol spraying reagent. Standard synephrine was used for comparison.

Developing solvent systems: 100 ml of solvent system, n-butanol: acetic acid :water (4:1: 2.2) was placed in a glass tank (22.5gm×22cm×7cm) and covered with a glass lid and allowed to stand for 45 minutes before use. A small amount of extract (1 mg dissolved in 1 ml solvent) was applied with standard sample (1mg/ml) to TLC plates manually, using capillary tubes.
Table 3: Description of p-synephrine\cite{5}

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC NAME</td>
<td>4-[1-hydroxy-2-(methylamino)ethyl]phenol</td>
</tr>
<tr>
<td>SYNONYM</td>
<td>Oxedrine, Sympatol, Sympaethamine, Parasympatol</td>
</tr>
<tr>
<td>DESCRIPTION</td>
<td>sympathomimetic α-adrenergic receptor agonist</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>( \text{C}<em>9\text{H}</em>{13}\text{NO}_2 )</td>
</tr>
<tr>
<td>Molar mass</td>
<td>167.21 g/mol</td>
</tr>
<tr>
<td>Appearance</td>
<td>Off-white solid powder</td>
</tr>
<tr>
<td>Density</td>
<td>1.159 g/cm(^3)</td>
</tr>
<tr>
<td>Melting point</td>
<td>187 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>341.1 °C at 760 mmHg</td>
</tr>
<tr>
<td>Purity</td>
<td>( \geq 98% )</td>
</tr>
<tr>
<td>Storage</td>
<td>2 -8</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water to 100 mM (with warming), soluble in ethanol to 10 mM (with warming).</td>
</tr>
</tbody>
</table>

**Biosynthesis**

The biosynthesis of synephrine in *Citrus* species is believed to follow the pathway: tyrosine \( \rightarrow \) tyramine \( \rightarrow N\)-methyltyramine \( \rightarrow \) synephrine. This pathway differs from that in animals involving, octopamine \( \rightarrow \) tryamine \( \rightarrow \) octopamine \( \rightarrow \) synephrine.\cite{6}

Table 4: Human biosynthesis pathway for trace amines and catecholamine

\[\text{AAAH} \rightarrow \text{Aromatic L-amino acid hydroxylase} \quad \text{COMT} \rightarrow \text{Catechol-O-methyl transferase} \quad \text{DBH} \rightarrow \text{Dopamine } \beta \text{ hydroxylase} \quad \text{AADC} \rightarrow \text{Aromatic L-amino acid decarboxylase} \quad \text{PNMT} \rightarrow \text{Phenylethanolamine N-methyl transferase.}\]
Synthesis

Synthesis of synephrine was carried out by the O-benzyolation of p-hydroxy-phenacyl chloride which is obtained by the reaction of phenyl benzoate and chloro acetyl chloride, followed by reaction of the resulting O-protected chloride with N-methyl benzylamine to give an amino ketone.

This intermediate was then hydrolysed with HCl/ alcohol and then reduced catalytically to give (racemic) synephrine.[7]

A later synthesis began with the O-benzylolation of p-hydroxybenzaldehyde, followed by a Reformatsky reaction of the protected aldehyde with ethyl bromoacetate/Zn to give the expected β-hydroxy ester. This intermediate was converted to the corresponding acyl hydrazide with hydrazine, then the acyl hydrazide reacted with nitric acid ultimately yielding the p-benzyloxy phenoxazolidone. This was N-methylated using dimethyl sulphate, then hydrolyzed and O-debenzylated by heating with hydrochloric acid to give racemic synephrine.[8]
Structural activity relationship

- p- synephrine possesses a OH group on the benzene ring. This substituent increases the polarity of the benzene ring which reduces BBB penetration. Furthermore, it possesses a -OH substituent on the β -carbon. The addition of the para hydroxy group on the p-synephrine molecule, as well as the lack of the additional methyl group, greatly decreases the lipid solubility of p-synephrine as compared to ephedrine, a phenylpropanolamine derivative, having a methyl group on the α -carbon of the side chain and no para-substituted hydroxyl group. As a consequence, p-synephrine exhibits little or no CNS and cardiovascular stimulation. p- synephrine may act locally on the cardiovascular system. The hydroxyl group in the p-position also enhances its interaction with phase 2 metabolism and also favours complete elimination. Finally, p-synephrine possess a secondary terminal amine which generally balance in favour of β- adrenergic affinity Vs α -adrenergic in comparison to its norsynephrine analogue.

- The replacement of N-methyl group with hydrogen gives octopamine.
- Replacement of the β-hydroxy group in synephrine by a hydrogen atom gives N-methyltyramine; which essentially precludes it from adrenergic receptor agonism.
- Replacement of the synephrine phenolic 4-OH group by a –H gives halostachine.
- If the synephrine phenolic 4-OH group is shifted to the meta-, or 3-position on the benzene ring, the compound known as phenylephrine (or m-synephrine, or "Neo-synephrine") results-a nasal decongestant.
- If the same group is shifted to the ortho-, or 2-position on the ring, o-synephrine results.
- Addition of another phenolic–OH group to the 3-position of the benzene ring produces the neurotransmitter epinephrine.

Halostachine was demonstrated to be 19% as effective as epinephrine in activating the β2 receptor and m-synephrine 24% effective.\[9,10\]

**Mechanism of action**
The fat cells have two different types of receptors for catecholamines.
- Alpha receptors (α1, α2)
- Beta receptors (β1, β2, β3)
Adrenoreceptors endogenously exist in white and brown adipose tissues and muscle, and activation increases lipolysis and lipid metabolism.

These receptors are diametrically opposed in function $\alpha$-receptors hinder fat mobilization and $\beta$ receptors activate it. Thus, fat cells with a high amount of $\beta$ receptors are relatively easy to burn (shrink) while $\alpha$-receptors doesn’t.

$\beta_3$ adrenoreceptor mediated lipolysis in adipocytes is mediated via the adenyl cyclase/cAMP/protein kinase A, signaling cascade with downstream production of nitric oxide (NO). Thus, $p$-synephrine may act as a $\beta_3$ adrenoreceptor agonist, resulting in increased metabolic rate and lipolysis as well as promote opening of $\text{Ca}^{2+}$ channels, exhibiting hypoglycemic and insulin stimulatory properties.

As $p$-synephrine exhibits poor binding affinities for $\alpha$, $\beta_1$, $\beta_2$ adrenoreceptor subtypes relative to norepinephrine, $m$-synephrine, ephedrine, and other amines it exhibits little or no cardiovascular activity when administered orally either alone or in the form of products containing multiple ingredients. \[10, 11\]

### Pharmacokinetics

**Table 5: Pharmacokinetics** \[10\]

<table>
<thead>
<tr>
<th>Absorption</th>
<th>Well absorbed peak plasma concentration is 1-2 hours.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>does not cross blood brain barrier</td>
</tr>
<tr>
<td>Metabolism</td>
<td>metabolized via phase II metabolism to give octopamine</td>
</tr>
<tr>
<td>Elimination</td>
<td>through urine</td>
</tr>
</tbody>
</table>

**Dose**

32 mg/ day in treating obesity. \[12\]

**Contraindications**

$p$-synephrine is avoided in pregnancy, lactation and in patients with hypertension, tachyarrhythmia, type 2 diabetes mellitus or narrow angle glaucoma. \[12\]

**Adverse effects**

- Palpitations
- Increase in blood pressure
- Increased risk of stroke
- Increased risk of adverse cardiac events like cardiac arrhythmias. \[13\]

**Drug interactions**

Bitter orange may inhibit intestinal CYP3A4 and intestinal efflux and may interact with numerous drugs, including

- Anxiolytics

- Antidepressants phenelzine (Nardil), tranylcypromine (Parnate)
- Antiviral agents
- Dextromethorphan
- GI prokinetic agents
- Calcium channel blockers, vasoconstrictors amiodarone (Cordarone), disopyramide (Norpace), dofetilide (Tikosyn), ibutilide (Corvert), procainamide (Pronestyl), quinidine, sotalol (Betapace).

- Midazolam (Versed)
- Dextromethorphan (Robitussin DM, and others)
- Felodipine (Plendil) \[14\]
- Indinavir (Crixivan)
- Lovastatin (Mevacor), ketoconazole (Nizoral), itraconazole (Sporanox), fexofenadine (Allegra), triazolam (Halcion), and many others.

- Diethylpropion (Tenuate), epinephrine, phenetermine (Ionamin), pseudoephedrine (Sudafed).
DISCUSSION
Thermogenic effects of p-synephrine alone and in conjunction with the flavonoids naringin and hesperidin.

The literature survey showed that in a double-blinded, randomized placebo controlled study. Approximately 44% of the subjects consumed a bitter orange/p-synephrine only product, while the remainder consumed a complex product that contained multiple ingredients in addition to p-synephrine. In general, bitter orange extract alone (p-synephrine) or in combination with other herbal ingredients did not produce significant adverse events as an increase in heart rate or blood pressure, or alter electrocardiographic data, serum chemistry, blood cell counts or urine analysis. The group receiving p-synephrine (50 mg) alone exhibited a 65 kcal increase in RMR, 600 mg naringin with 50 mg p-synephrine resulted in a 129 kcal increase in resting metabolic rate (RMR) 100 mg hesperidin in addition to the 50 mg p-synephrine plus 600 mg naringin, the RMR increased by 183 kcal, when compared to the placebo group. p-Synephrine alone as well as in combination products were shown to increase RMR and energy expenditure, and thus assisted weight management when given for 12 week.[15]

The effects of supplementation with p-synephrine alone and in combination with caffeine on free-weight resistance exercise performance.

Each subject was randomly assigned to a treatment sequence consisting of use of 3 supplements: p-synephrine (A; 10mg), p-synephrine + caffeine (B; 100mg of p-synephrine + 100mg of caffeine), or a placebo. On each day protocol, subjects reported to lab consumed a supplement, sat quietly for 45 min, performed the resistance exercise protocol, and rested for 30 min post exercise. Performance (repetition number, force, velocity and power), blood lactate and exertion ratings were collected each time. These results indicated the supplementation with p-synephrine and in combination with caffeine can enhance local muscle endurance during resistance exercise without increasing the blood lactate level.[16]

The effects of a bitter orange extract containing product on body fat loss, lipid levels, safety and mood in 20 overweight adult subjects.

The product consumed on a daily basis contained 975mg, 6% p-synephrine, 528mg caffeine and 900mg St. John’s wort. All subjects followed an 1800 kcal/day diet. After 6 weeks, the treated group lost small but significant amounts of body weight (1.4 kg) and body fat (2.9%). No significant changes in blood pressure, heart rate, serum and urine analysis were noted and no changes were observed in the results of the mood states questionnaire for fatigue or vigor. The treated group also experienced a significant increase in basic metabolic rate as compared to the placebo group.[17]

The stereochemical and pharmacological differences between naturally occurring p-synephrine and synthetic p-synephrine.

p-Synephrine, the primary protoalkaloid in Citrus aurantium (bitter orange) and some other Citrus species, exists in nature in the l- or [R-(+)]- enantiomeric form, whereas synthetic p-synephrine is a racemic mixture of the l- and d- enantiomeric forms. Based on receptor binding, the synthetic form is believed to exert approximately half the pharmacological activity of the naturally occurring protoalkaloid. This difference occurs because the d- or [S- (+)]-form provides little or no binding to adrenergic receptors in contrast to the l-form. Receptor binding studies also provide an explanation for the differences in pharmacological effects between the isomers p-synephrine and m-synephrine. Also the dl mixture of a drug will have less activity.[18]
increased epinephrine excretion by 2.4 fold. An increase in the thermic effect of food in women suggests a diminished sympathetic nervous system response to meals, because with bitter orange an increase was seen only in women.\textsuperscript{18}

Seifert et al. conducted a study on the effects of an herbal blend on energy expenditure in mildly obese subjects. The product contained 13 mg p-synephrine (as Advantra Z\textsuperscript{®}), 176 mg caffeine (as guarana), and 55.5 mg of a green tea extract per capsule. The study involved 14 females and 9 males in a placebo-controlled, crossover design. Subjects ingested one capsule with each of three meals on day one of treatment, and one more capsule on the morning of the second day. Data were collected 60 min after the last administration of the product. The results demonstrated that from pre-test on day one to post-test on day two, caloric expenditure increased by approximately 8% following ingestion of the product. Oxygen uptake increased from 230 to 250 ml/minute following treatment. No differences were observed in heart rate or blood pressure following treatment. This was an acute study which did not provide information on long-term effects, but did demonstrate an increase in energy expenditure.\textsuperscript{19}

**Weight loss with an antioxidant and tissue protective effect.**

This was assessed by treating mice orally (by gavage) daily with bitter orange extract (7.5% p-synephrine) at doses of 30, 150, 300mg p-synephrine/kg and observed for 1 min at 15 min intervals. These doses are very high relative to doses consumed by humans in dietary supplements. Mice were observed for the presence of respiratory, digestive and neurological alterations. The number of deaths and weights was noted in each 24 hours. Animals that died were immediately necropsied and analysed. After 14\textsuperscript{th} day, mice that survived were sacrificed and necropsied. The result suggest a beneficial effect with respect to weight loss without adverse effects while also providing an antioxidant and tissue protective effect based on the increased glutathione levels and increased catalase activity with no increase in lipid peroxidation.\textsuperscript{20}

**The binding activities of the m- and p- isomers of synephrine and octopamine to rat aorta α\textsubscript{1} and rabbit vein α\textsubscript{2} adrenoreceptors.**

The binding of m-synephrine to α\textsubscript{1} and α\textsubscript{2} adrenoreceptors was 6-fold and 150-fold less respectively than norepinephrine. Furthermore, p-synephrine and p-octopamine were 1000-fold less active than norepinephrine in binding to these two receptors, again demonstrating that m-synephrine much more actively binds to these receptors than p-synephrine. Based on these receptor binding studies, p-synephrine would be expected to exert little vasoconstriction and therefore, little or no effect on blood pressure as compared to m-synephrine or norepinephrine.\textsuperscript{21}

The synephrine and octopamine concentrations in Seville orange juice (sour) and its cardiovascular effects in normotensive humans.

The concentration of p-synephrine in the orange juice was 56.9 ± 0.52μg/ml while octopamine was not detected. This was done by high performance liquid chromatography. Subjects consumed 8 ounces of orange juice (approximately 13mg p-synephrine) and water in a crossover fashion followed by a repeated ingestion 8 hours later. Hemodynamic (heart rate, diastolic and mean arterial pressure) measurements were followed.12 normotensive subjects of mean weight 82.2 ± 19 kg and 61.4±3 kg for male and female respectively were considered. All volunteers were nonsmokers or had no history of hypertension or taking nonprescription analysis and neither the females was taking contraceptive during the study periods.

The authors concluded that the in ingestion of orange extract by normotensive subjects is expected to be safe. Individuals with severe hypertension, tachyarrhythmias, and narrow–angle glaucoma and monoamine oxidase inhibitor recipients should avoid this. Persons taking decongestant containing cold preparations should also refrain from juice intake. The warning was based on the erroneous assumption that the form of synephrine present in the orange juice was m-synephrine.\textsuperscript{22}

The physiological and metabolic effects of product containing bitter orange, guarana and green tea extracts in 20 overweight individuals at rest and during treadmill.

Subjects received either the placebo or the product and were followed for 7 hours or exercised on a treadmill for 60 min. The product had no effect on ATP utilization under resting or exercise conditions relative to control. However, a 30% increase in carbohydrate oxidation was observed. Fatty acid oxidation to ATP decreased while plasma levels of fatty acids increased in response to the product.\textsuperscript{23}

CONCLUSION

Bitter orange (citrus aurantium) extract and its protoalkaloid p-synephrine are used widely in weight loss managements and sports performance products after the ban of ephedra. The naturally occurring p-synephrine in citrus and as a trace amine produced in the human body [l-enantiomer] is more potent and pharmacologically active than synthetically produced [d-form].

\textit{p}\textsuperscript{-}synephrine is a β agonist compound. It can increase the weight loss via

- Increasing the increasing lipolysis and basal metabolic rate over an extended period of time.
- Inhibiting the activity of fat cells receptors (α) that prevent fat mobilization.
- Increasing the thermic effect of food, or the “energy cost” of metabolizing food.
Several studies shows that this metabolic boost can be significantly increased by combining synephrine with two other molecules found in the bitter orange fruit: naringin and hesperidin. Furthermore, studies show that synephrine works synergistically with caffeine. It also increased the thermic of food especially in women. Overall, usage of p-synephrine appears to be quite safe and free of most adverse side effects.

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REFERENCES