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Evaluation of chemical characterization and antihyperlipidemic potential of orange peel powder *(Citrus sinensis)* in male human subjects

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Abstract: *Citrus sinensis* is an important member of the genus *Citrus* which contains phenolic compounds and bioflavonoids which have antihyperlipidemic and antiatherogenic effects. It also has the potential to reduce oxidative stress. To investigate the antihyperlipidemic effect of orange peel powder was encapsulated and analyzed in hyperlipidemic patients. Results showed that it contains moisture (12.2%), ash content (7.9%), crude fat (0.78%), crude protein (12.37%) and crude fiber (13.2%). Total phenolic content and total flavonoid content were observed as 163.17 mg and 17.23mg in quercetin equivalent per gram a dry weight basis. Furthermore, the Orange peel powder was given in the form of medicinal capsules to hyperlipidemia male subjects. The experimental groups (G_1 and G_2) were given orange peel powder in capsules 400mg/d to the G_1 group and 800mg/d to the G_2 group for the time of 45 days. The serum lipid profile of patients was measured before and after the experimental trial. The result showed that G1 and G2 showed a decrease in plasma lipid parameters and increased high-density lipoprotein content in blood substantially as compared to G_0. Thus, it was concluded from the results that orange peel powder depicts a significant impact on treating hyperlipidemia.

Keywords: Hyperlipidemia, orange peel powder, hypercholesterolemia, citrus, total phenolic content, total flavonoid content, minerals, LDL, triglycerides.

INTRODUCTION

A great deal of evidence has been collected over twenty years that there is a close association between hyperlipidemia and atherosclerosis. An elevated lipid profile leads to clinical manifestations of atherosclerosis including coronary heart disease, stroke and other clinical conditions. Acute pancreatitis is found to be linked with increased triglycerides level (Eaton, 2005). Hyperlipidemia has prevailed around the world, but it is more common among western people. Hyperlipidemic humans are at higher risk of developing coronary heart disease than those with normal lipid profiles (Karr, 2017). The lipid profile comprises total cholesterol, triglycerides, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (Nordestgaard, 2017). Among these LDL is involved in atherogenesis which is plaque formation in the arteries. The risk of atherogenesis and coronary heart disease can be reduced by lowering the LDL levels (Jain *et al*., 2007). An incessant member of the genus *Citrus* and family *Rutaceae*; *Citrus sinensis* which is commonly known as orange or sweet orange is among the world's largest cultivated fruit plants with an annual production of 70% of all *Citrus* species (Flamini *et al*., 2003). *Citrus sinensis* is grown in warm regions of the world and it is native to Asia (Steduto *et al*., 2012). Flavonoids are dietary polyphenols in *C. sinensis* that have gained the attention of researchers in lowering elevated lipid profiles. Naringin is an active bioflavonoid found in *C. sinensis* that possesses antihyperlipidemic, anti-inflammatory and antiatherogenic properties along with other health benefits (Mallick & Khan, 2016). It also contains fiber and possesses a great ameliorative effect in lowering the elevated lipid profile which decreases the reabsorption of bile and enhances bile acid excretion in feces (Ling *et al*., 2020). All these properties have made *C. sinensis* an important therapeutic plant with tremendous medicinal properties. The objective of this

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study was to investigate the anti-hyperlipidemic effect of *C. sinensis* in hyperlipidemia patients. The study also investigated the chemical characterization of *C. sinensis* peel including proximate value, mineral analyses and phytochemical determination.

MATERIALS AND METHODS

Collection and preparation of raw material

To identity required genome of *Citrus sinesis*, assistance was taken from botanist of National Institute of Agriculture and Biotechnology. Oranges (*Citrus sinensis)* was purchased from the local market. All the dirt and debris were removed. Oranges were washed and then peeled off. The surface of the orange peel was cleaned with 70% alcohol and then washed with sterilized distilled water. It was dried in a laboratory hot air oven at 50°C overnight and then finely ground into powdered form by using an electric grinder (El-Beltagi *et al*., 2022). This refined powder of orange peel was stored in air-tight containers and stored at room temperature (El-Shazly *et al.*, 2017).

Chemical composition of orange peel powder

Proximate Profile of Orange (C. sinensis) peel Powder

Orange (*Citrus sinensis)* powder was analyzed for the following proximate profile including moisture content, ash, carbohydrates, crude protein, crude fiber, and nitrogen-free extract (NFE) according to the AOAC method (Garcia-Amezquita *et al.,* 2018).

Minerals determination

The following minerals zinc, magnesium, iron, phosphorus, potassium, sodium and copper were analyzed by using atomic absorption spectrometry (Hernández *et al*., 2005).

Phytochemical features of orange peel powder

Phytochemical analysis of orange peel powder investigated for determination of total phenolic content (TPC) and total flavonoid content (TFC) in methanol extraction. The amount of TPC AND TFC were measured in mg of quercetin equivalents (QE) per g of dry weight basis of the sample (M'hiri *et al*., 2015).

Investigation of therapeutic potential of orange peel powder against hyperlipidemia in human subjects

Selection of hyperlipidemic male subjects

45 hyperlipidemic male subjects of age 40-65 years were randomly selected from the general community.

Exclusion criteria

Following subjects were not included in the study:

- Patients taking antihyperlipidemic drugs.
- Patients with other chronic complications like cardiovascular diseases along with hyperlipidemia.

Inclusion criteria

All hyperlipidemic male subjects went through blood biochemical analysis by checking their lipid profile at the start of the study was included.

Study duration

The study was conducted for 45 days.

Treatment groups and treatment plan

Male subjects were divided into three groups of 15 subjects each, with the following conditions. The control group was given no treatment. Treatment group-I and treatment group II were given doses of orange peel powder in capsulated form (Papagianni *et al*., 2021).

Ethical approval

Ethical approval was taken from the biosafety committee of the institute Ref. #Ec-273.

STATISTICAL ANALYSIS

Descriptive statistical analysis using the two-sample t-test under a Completely Randomized Design (CRD) was carried out to investigate the level of significance ($p<0.05$). All statistical analyses are done with IBM SPSS Statistics 20.

RESULTS

The study was conducted to investigate the hypolipidemic therapeutic potential of orange peel powder against hyperlipidemia to check its effectiveness in lowering lipid profile.

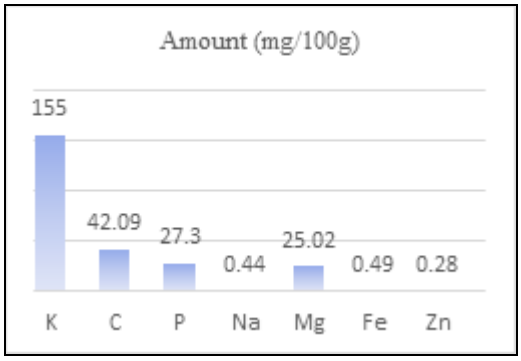

Fig. 1: Mineral analyses of orange peel

Proximate composition of orange peel powder

The orange powder was investigated for varied characterizations, such as moisture, ash, crude fiber, crude proteins, fat and nitrogen-free extract (NFE) depicted in table 2.

Mineral analysis

In the current study, orange peel powder was also analyzed for the determination of mineral content

Table 1: Treatment groups and treatment plan

Treatment Groups	Title	Treatment
G_0	Control group	No treatment
G_1	Treated group 1	Capsule with orange peel powder of 400 mg
G_2	Treated group 2	Capsule with orange peel powder of 600 mg

including calcium, potassium, iron, zinc, phosphorus, magnesium and sodium depicted in table 3.

Phytochemical characters of orange peel powder

The orange powder was analyzed for TPC and TFC analysis. The orange peel powder carried a significant amount of TPC and TFC depicted in table 3.

Investigation of antihyperlipidemic potential of orange peel powder

The objective of this study was to investigate the nutraceutical and therapeutic potential of orange peel powder in lowering the elevated lipid profile in 45 hyperlipidemic male human subjects. Participants (n=15) of the control group (G_0) were given no treatment while participants of treatment group-I (G_1) and treatment group- II (G_2) were given orange peel powder in doses of 400mg/d and 600mg/d in capsulated form respectively for 45 days. Before the commencement and after the standstill of the trial, blood samples were gathered from each subject for analysis of lipid profile (HDL, LDL, triglyceride, total cholesterol).

Table 1: Mean ± S.D for proximate % composition of orange peel powder on dry weight basis

Proximate analysis	Composition (%)
Moisture	12.2±0.31
Ash	7.9±0.63
Nitrogen Free Extract	53.5±2.71
Crude protein	12.37±0.42
Crude Fat	0.78±0.32
Crude fiber	13.2±1.02

Table 2: Mean ± S.D for phytochemical analysis of orange peel powder on dry weight basis

Antioxidant	Antioxidant content (mg QE/g)
TPC	163.17±3.1
TFC	17.23±0.4

Table 3: Mean ±S.D for serum cholesterol level of male subjects

Span	G_O	G_1	G_2
0 day	234.0± 10.48	256.23± 7.83	244.32± 4.52
45th day	241.70± 12.44	229.12± 6.42*	204.34± 5.36*

G_0 = No Orange peel powder, G_1=400mg/d of orange peel powder, G_2= 600mg/d of orange peel powder. Results were taken significant at $p<0.05$.

Table 4: Mean ± S.D for triglycerides level in male subjects

Span	G_O	G_1	G_2
0 day	171.30± 31.13	167.20± 24.67	173.20± 78.76
45th day	183.70± 34.43	123.30± 58.94*	114.40± 36.62*

G_0 = No Orange peel powder, G_1=400mg/d of orange peel powder, G_2= 600mg/d of orange peel powder. Results were taken significant at $p<0.05$.

Changes in serum cholesterol level of male subjects

The investigation showed a significant ($p<0.05$) reduction in serum cholesterol levels in response to orange peel powder capsules in both treatment groups. Group-I showed a reduction in the total cholesterol level from 256.23±7.83mg/dl to 229.12±6.49 mg/dl while group II showed a reduction in serum cholesterol level from 244.32±4.52mg/dl to 204.34±5.36mg/dl. Orange peel dose of 600mg/day in G_2 showed better results than 400 mg/d in G_1. On the other hand, G_0 shows a momentous increase in cholesterol levels.

Table 5: Mean ± S.D for HDL level in male subjects

Span	G_O	G_1	G_2
0 day	39.70±5.75	41.40±7.66	42.67±4.87
45th day	38.600±5.76	47.00±7.08*	48.90±5.31*

G_0 = No Orange peel powder, G_1=400mg/d of orange peel powder, G_2= 600mg/d of orange peel powder. Results were taken significant at $p<0.05$.

Table 6: Mean ±S.D for LDL level in male subjects

Span	G_O	G_1	G_2
0 day	140.60± 11.19	139.60± 9.35	117.00± 13.80
45th day	137.20± 16.17	121.60± 14.14*	94.20± 11.40*

G_0 = No Orange peel powder, G_1=400mg/d of orange peel powder, G_2= 600mg/d of orange peel powder. Results were taken significant at $p<0.05$.

Reduction in serum triglycerides level of male subjects

Both experimental groups showed significant reduction in triglyceride levels from 167.20±24.67mg/dl to 123.30± 58.94mg/dl in G and from 173.20±78.76mg/dl to 114.40±36.62mg/dl G_2. Although on the other hand, G_0 manifests no significant change in the TGC level as shown in table 5.

Positive changes in the serum HDL levels

As compared to the G_0 group, HDL was increased in the experimental group G_1 and G_2. G_1 shows a change in HDL from 41.40±7.66mg/dl to 47.00±7.08mg/dl and G_2 showed an increase in HDL from 42.67±4.84mg/dl to 48.90±5.31 mg/dl. While on the other hand, G_0 showed no significant change in the level of serum HDL as compared to G_1 and G_2 table 6.

Reduction in the serum LDL Levels

A significant decline was observed in the experimental group after taking orange peel powder for 45 days. G_1 and G_2 showed a reduction in the level of serum LDL from 139.60±9.35mg/dl to 121.60±14.14mg/dl in G_1 and from 117.00±13.80mg/dl to 94.20±11.40mg/dl in G_2. On the other hand, G_0 showed no significant changes in the LDL level as compared to the groups taking orange peel powder as shown in table 7.

DISCUSSION

This study investigated the medicinal effect of orange peel against elevated lipid profile. Proximate analysis, mineral determination and phytochemical analysis were also performed to evaluate the characteristics of orange peel. Results determination of proximate composition was momentous in deciding the worth of raw materials being used. These six constituents built up the whole composition of orange peel powder majorly and these findings were close to previous studies (M'hiri *et al*., 2015). The mineral composition findings had shown that potassium and calcium were found in greater amounts than others and these findings were consistent with a previous study on the mineral content of citrus peel and pulp (Czech *et al*., 2020). Calcium found to be an important ion, and it regulated many biological functions in the body. Calcium was also involved in promoting the hormones involved in fat metabolism and it also helped in dealing with obesity (Yamaguchi, 2010).

Phenolic chemicals were vital components of plants that possess antioxidant properties and control oxidative stress in plants. These phenolic compounds possessed hydroxyl groups which scavenge free radicals. The phenolic compounds were extracted and investigated for their antioxidant activity. Due to this antioxidant potential orange peel powder showed the potential of lowering lipid profile and antiatherogenic effect. Orange peel reduced the elevated cholesterol, triglycerides and LDL. Orange peel increased the HDL. A study reported that orange peel contained naringin, a bioflavonoid in orange and other citrus fruits. Naringin possessed hypolipidemic properties. Naringin contained properties of strong antioxidant which helped in lowering the oxidative stress and reduced LDL in return. These findings were also observed in another study that investigated the antihyperlipidemic effect of orange peel (Abdo *et al*., 2022).

Reduction in elevated lipid profile was correlated to another study that was conducted to see the antihyperlipidemic effect of orange peel extract in high fat diet induced hyperlipidemia in rats (Pallavi M *et al*., 2021). Results reported an increase in HDL level in the treatment group and reduced the triglycerides levels. These results were also similar in mice lipid profile both normal and induced oxidative stress that further make this study evident (Al-Jowari & Moosa, 2013). These results correlated with another study that reported reduction in LDL level in response to orange peel extracts however, the reduction in LDL level of this study showed better reduction that was might be due to minor difference in using peel and peel extract (Pallavi M *et al*., 2021). A study showed that investigated the effect of orange peel extract in humans lipid profile and blood sugar levels slightly different results which reported reduction in triglycerides levels but rest of the parameters did not improved much which was contrary to the results of this study (Papagianni *et al*., 2021). This contradiction might be due to the use of extract but not peel which can be further investigated.

CONCLUSION

The increasing prevalence of hyperlipidemia is due to unhealthy and sedentary lifestyles. People are paying more attention to plant-based therapies for hyperlipidemia due to lesser side effects. A current study had investigated the therapeutic effect of orange peel powder against hyperlipidemia. Orange peel powder had shown ameliorative and nutraceutical properties in lowering the elevated lipid profile parameters. Both doses of 400mg/d and 600mg/d were proven beneficial in lowering the lipid profile parameters including triglycerides, cholesterol, and LDL levels.

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