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Garden Cress Seed Jellies: A Novel Nutraceutical for Health and Wellness

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Abstract

The demand for nutraceutical is increasing day by day. Nutraceutical products are recognized not only for their better health outcomes to reduce the risk of cancer, heart diseases, cataracts, menopausal symptoms, insomnia, anaemia, bronchial asthma, and gastrointestinal problems [1,2].

GC/Garden cress seeds act as a superfood that promote overall health and well-being by providing ample nutrition. It is a good source of iron, calcium, potassium, and vitamins that are required to maintain the homeostasis of our body [3].

GC seeds are also known to have anti-inflammatory, anticancer, antiasthmatic and antibacterial activities. Our project focuses on preparation of GC seed jellies as a nutraceutical. People of diverse age groups will be able to consume this nutraceutical, thus increasing patient compliance.

To understand the effect of these substituents on the biological activity a quantitative structure activity relationship (QSAR) study was performed using a data set of 41 compounds containing Spirocyclic Chromanes nucleus. Descriptors were generated using various free software's such as PADEL-Descriptor, Swiss ADME and OCHEM.

Introduction

Garden cress seeds biological name is ***Lepidium sativum*** also known as **Garden Cress seeds** or "**Chandrasura**". Garden cress comes under the family ***Brassicaceae***.

Lepidium sativum main feature is that it can be grown in any type of climate and soil condition, and has an ability to tolerate slight acidity in soil and can be grown like white mustard. [4, 5].

MORPHOLOGY OF GARDEN CRESS SEEDS:

Shape: Oval, pointed, triangular at one end

Color: Reddish brown to dark brown

Length: about 2-3 mm long and 1-1.5 mm wide

Seed coat swells by soaking them into the water and then gets covered with transparent, colorless, mucilage with mucilaginous taste.

TAXONOMICAL CLASSIFICATION:

Kingdom: Plantae

Division: Magnoliophyta

Phylum: Tracheophyta

Class: Magnoliopsida
Order: Brassicales
Family: Brassicaceae
Genus: Lepidium
Species: Lepidium sativum [4].

CHEMICAL CONSTITUENTS:

Garden Cress seeds are composed of folate, iron, vitamin A, vitamin C, vitamin E, and vitamin B-complex like Carotene, riboflavin, thiamine, niacin. These seeds are also a good source of iron, calcium, magnesium, and protein, making them a great addition to a healthy diet. The seeds are also a rich source of acids like arachidic acid, folic acid, ascorbic acid, and beta carotene which helps us to improve our immune system. The seeds are also a rich source of chemicals like oestrogen which helps to regulate Menstruation and stimulate milk production for mothers. The oil of these seeds has a balanced amount of (MUFA) monounsaturated fatty acids (37.6%) and PUFA (Polyunsaturated fatty acids) 46.8 %. It also shows antioxidant, anti-inflammatory, anticancer, antiasthmatic and antibacterial activity because of the presence of antioxidants like eugenol and vitamin A, E, which helps to protect the cells from damage by free radicals [6]. Phenolic compounds like gallic, ellagic, protocatechuic, chlorogenic, coumarin and Iso-ferulic play an important role in improving the human health by participating in the antioxidant defence system against free radical generation. A wide variety of amino acids (47.08%) are present in GCS including essential amino acid histidine, threonine, arginine, valine, methionine, phenylalanine, and isoleucine.

The quantitative analysis of *Lepidium sativum* seeds showed that the seeds contained protein (24.2±0.5%), lipids (23.2±0.2%), carbohydrates (30.7±1.2%), fiber (11.9±0.4%), ash (7.1±0.1%), moisture (2.9±0.1%), alkaloids (0.40%), flavonoid 0.42%), saponin (2.8%), tannin (0.61%) and phenol (0.004%).

Wholemeal, endosperm and bran were analysed for chemical composition. The whole meal, endosperm and bran contained 22.5, 27.7 and 12.6% protein, 27.5, 33.1 and 6% fat, 30, 13.6 and 75% dietary fiber, and 1193.00, 945.15 and 1934.57 potassium, respectively. The most abundant amino acid was glutamic acid (19.3%), the essential amino acid, leucine was the highest (8.21±0.01%) and methionine was the lowest (0.97±0.02%). The major fatty acid was linolenic acid (30.2%) and the lowest was erucic acid (3.9%). Bran has high water holding capacity and high dietary fiber [6,7].

Table no 1 Nutrition value of 100gm of garden cress seeds

Nutrient	**Percentage%**
Dietary fibers	30
Proteins	22.5
Fats	30

Table no 2:

Nutrient	Nutritional value
Proteins	2.6g
Vitamin A	346 micrograms
Calcium	81mg
Folic acid	80 micrograms
Carbohydrates	5.5g
Vitamin C	69mg
Fats	0.7g
Iron	1.3mg
Food fibers	1.1g

MEDICAL PROPERTIES OF GARDEN CRESS SEED:

GC contains high percent of vitamin-C. It helps to maintain the bones, skin, and the healthy gums. It also ensures to cover the body needs in Iron. As perhaps it improves the body ability to absorb iron from the eaten foods.

- **Treat anaemia:** Contain a high amount of iron and folic acid. In the human blood it helps in increasing the standard of Haemoglobin. It helps to treat the deficiency of iron which leads to anaemia. GC contains a high amount of vitamin C and iron that can be used for treating anaemia.
- **Boots the secretion of breast milk:** GC has high nutritional value that is beneficial for the nursing mother. It helps in nursing mothers for the continued flow and production of the Breast milk containing the high tenor of protein and iron, because the presence of protein as whey remains in a liquid and is easier to digest**.**
- **Helps to treat diabetes:** The phytoconstituents in GC seeds are efficient in treating diabetes.
- **Treat constipation and stimulate gastrointestinal function:** Used for treatment of sore throat, asthma, headaches, and coughing. It is advised to the people with bronchitis because it contains the characteristics of bronchodilator.
- **Treats menstrual cycle disorders:** Regulation of menstrual cycle in women is an important factor for knowing the correct time or approximate time of pregnancy. The seeds are of great help in women with less flow or irregular flow during menstruation. The presence of phytochemicals like estrogens like in maintaining hormone levels in women and regulating the menstrual cycle.
- **Treats high blood pressure:** Garden cress seeds are composed of antihypertensive as well as diuretic activities which help in treatment of blood pressure.
- **Protects against cancer:** Garden cress seeds contain antioxidants like vitamin A and Vitamin E which helps to protect the cells from free radicals. It also stops the growth of tumours by production of certain enzymes and contributes to killing breast cancer. Benzyl isothiocyanate (BITC) effectively suppresses

growth of cultured human breast cancer cell. BITC treatment caused rapid disruption of the mitochondrial membrane potential.

- **Strengthens immune system:** Vitamins present in garden cress seeds are good immunity boosters which improves the functions of white blood cells. These vitamins also prevent the bacteria and viruses from entering the sensitive mucous membrane of the body like eyes, nose, mouth, throat, lungs, and stomach.
- **Benefits for Garden Cress for hair:** Due to the presence of vitamin C and antioxidant activity, the seeds help to treat damaged hair, prevent hair fall, and get healthy and thick hair [8,9].
- **GC seed intake improves the nutritional status of growing children and adolescent girl because:** It is a high energy food, providing 454 Kcal per 100g.
- It is rich in protein, containing 25.3g of protein per 100g.
- It is a good source of fat, having 24.5g of fat per100g [10].
- It is the richest source of iron, providing 100mg of iron per 100g [11].

Sweet lemon oil was used as a flavouring agent and pectin from sweet lemon was used as a gelling agent. Sweet lemon is a group of citrus hybrids that contain low acid pulp and juice. They are hybrids often like non-sweet lemons or limes, but with less citron parentage [12]. Although synthetic flavours are widely used in the food industry, flavours derived from natural sources have gained much importance. Citrus essential oils are used in many food industries as flavouring ingredients. Essential oils are mainly recovered from citrus peel, and volatile and semi-volatile components account for 85–99% of the total fraction with over 200 compounds. In addition to their extensive utilization as flavouring agents in food and medicine, essential oils also exhibit antibacterial, antifungal, and insecticidal properties [15, 16].

Sweet lemon has high vitamin C content, which can relieve inflammation and swelling. It also improves calcium absorption, stimulates bone formation in cells, and promotes overall bone health. The constituents of sweet lemon essential oil (EO) have different biological and medical properties [13].

Nata de coco, also marketed as coconut gel, is a chewy, translucent, jelly-like food produced by the fermentation of coconut water [14]. It is a complementary food derived from coconut water. Bacteria are used for the process, then it goes to the fermentation stage with Prebiotics, which forms a Gel or Cellular Membrane, with a high index of microscopic dietary fiber "Nano Fibers", very strong and resistant. Because it is rich in fiber, nata de coco is good for digestion.

Potential of garden cress seed in functional food development

Various food products are reported in the literature. Few examples are Pinni, chikki, panjiri, laddu, cookies, biscuits, Healthy Drink [19]. The major limitations of these food product are stability as in case of Pinni, laddus, health drinks etc. and acceptance as in case of cookies and biscuits.

Objectives and Rational

In numerous low-income countries consumption of animal source foods as a source of protein, carbohydrate and fat is minimal due to limited production, availability, and affordability of animal source foods. On the other side much attention has not been given for the multipurpose, under-utilized cereals like GC, despite can be used to formulate diverse functional foods due to the macronutrient and micronutrient (essential amino acids and fatty acids) content; apart from this it also promising source of bioactive (phytochemicals and polyphenols) compounds, which in deed potential to eradicate both under-nutrition.

Garden cress boosts immunity as it is loaded with flavonoids, folic acid and vitamins A, C and E. It has a rich content of iron and calcium. GS is an excellent food for improving the body's immunity and can help protect us from various infections and diseases [8,9]. Most of the reported products

Pharmaceuticals jellies have aesthetic appearance and pleasant taste than any other oral drug delivery systems. It has better organoleptic properties and patient compliance. Paediatrics and dysphagia patients can utilize the formulation more effectively and easily [17,8].

Summary of studies focusing on component identification and isolation from various GC seeds varieties including wider pharmaceutical applications like mucilage of the seed recommended. Although various traditional as well as in modern food products are reported, each one has some limitations. The aim of this project is to prepare nutritious jellies fortified with GCS or GCS oil. Jellies could be a more convenient, stable, cheap source of nutrients.

The jellies are made with gelling agent (agar, pectin), flavouring agent (sweet lemon oil), and sweetener (honey) and nata de coco. Pectin is the most important constituent of jelly [18]. Sweet Lemon oil is used because because it is a good preservative and improves calcium absorption [19].

Honey contains mostly sugar, as well as a mix of amino acids, vitamins, minerals, iron, zinc, and antioxidants. In addition to its use as a natural sweetener, honey is used as an anti-inflammatory, antioxidant, and antibacterial agent [20].

Coco de nata contains a considerable amount of fiber, which can help avoid conditions including constipation, haemorrhoids, coronary heart disease, appendicitis, colon cancer, diabetes, obesity, and more. Nata de coco is not only nutritious and fiber dense, but it also has a distinct flavour [21].

The GCS jellies could be a preferred source of iron for children, malnourished people, elderly, and patients who recovered from injury or illness.

Formulation aspect of Garden Cress seed jelly

Materials and methods

Table no.3

INGREDIENTS	BRAND NAME
Lepidium sativum (GC seeds)	Satyam
Citric acid	Rani
Propylparaben	
Agar Agar	Blue bird
Gelatine	Blue bird
Pectin	Blue bird
China Grass	Blue Bird
Carrageenan	Puramate
Honey	Dabur
Vanilla	Royal

Lepidium sativum seeds obtained from the local market. Citric acid was used to mask the unpleasant taste of the drug. Methyl Paraben and Propyl Paraben were used as preservatives. Experiments were carried out using solidifying agents/ jellying agents in different concentrations e.g. Agar Agar, Gelatin, Pectin, Chinagrass, Carrageenan. Honey was obtained from the market and used as a sweetener. Vanilla is used as a flavouring agent, butter paper

Apparatus:

Beaker, glass rod, water bath, Soxhlet apparatus, mixture grinder, spatula, TLC plate, round bottom flask, pH meter, Bunsen burner, tripod stand, wire gauze, tongs, graduated cylinder, funnel, weighing balance, dropper, test tube holder.

METHODOLOGY

PREPARATION OF JELLY:

Different formulations of jelly were prepared by using agar and gelatine as gelling agents in different concentrations as per given in the Formula table The gelling agents were tried initially
at different concentrations to achieve desired appearance, stiffness, and release. Citric acid was used to maintain the pH, propylene glycol was incorporated to enhance the softness and appearance of the jelly. Organoleptic agents were added to improve the aesthetic value of the jelly. Propylparaben was used as a preservative and honey was used as sweetening and bulking agents. The jellies are prepared by taking agar/ gelatine, propylene glycol, citric acid in a beaker and heated with continuous stirring to get solution form. Sweetening and flavouring agents were added and mixed thoroughly. The dispersion was transferred into moulds to avoid exposure to the outer environment. The formed jellies were wrapped in wax paper and stored in a dry place.

Table no. 4 FORMULATION TABLE (GCS)

INGREDIENTS	1	2	3	4	5	6
GC seeds	0.375g	0.375g	0.375g	0.375g	0.375g	0.375g
Gelling agent	0.4g (Agar)	0.2g(Gelatin)	0.6g(Pectin)	3g(Pectin)	0.3g(China grass)	0.3g(Carrageenan)
Citric acid	0.1g	0.1g	0.1g	1.5g	0.2g	0.2g
Propylene Glycol	0.3ml	0.3ml	0.3ml	4.5ml	0.1ml	0.1ml
Sugar syrup	6ml(honey)	6ml(honey)	6ml(honey)	4.5ml(honey)	9.9ml (6ml/7ml (honey/ liquid jaggery)	9.9 ml 6 ml/7 ml(honey)
Methyl paraben	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop
anilla	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop
Honey. Water	qs	qs	qs	qs	qs	qs

Ingredients	PECTIN	PECTIN + CARRAGEENAN	PECTIN+GELATIN
GC seed oil	2-3 drops	2-3 drops	2-3 drops
Gelling agent	0.3g pectin	0.3gpectin+0.3g carrageenan	0.3g pectin + 0.3gelatin/ 0,5g pectin+0.5g gelatine
Citric acid	0.1g	0.1g	0.1g
Propylene glycol	1ml	1ml	1ml
Sugar syrup	7ml (honey)	7ml	3.3ml

Dextrose	Not used	Not used	0.33g(0.3g pectin+0.3g gelatine)
Honey, water	Qs	Qs	Qs

Table No. 5 FORMULATION TABLE (GCS OIL)

Extraction of garden cress oil (GCS oil)

Soxhlet extraction of garden cress seed was carried out by following procedure. Ether and hexane were used as solvent for extraction.

Soxhlet extraction of GC seed oil

1. 25 g of GC seeds were weighed and ground to make a coarse powder
2. The powder was then packed in a muslin cloth and was placed in the thimble of Soxhlet apparatus.
3. 150 ml of petroleum ether was placed in a 250 ml round bottom flask and attached to a Soxhlet extractor and condenser.
4. The side arm is lagged with glass wool.
5. The heating mantle was used to maintain temperature of 30-50°C
6. The operation went on for almost one hour until the thimble portion of the apparatus got colorless.
7. After the desired amount of oil was extracted, the sample thimble was removed and the solvent received.
8. The collected oil transferred in the preweighed evaporating dish/ China dish and then after a specified amount of time, the Petri dish containing the oil was weighed and the reading for the yield was recorded.

Extraction procedure for sweet lime oil and pectin from sweet lime using Clevenger apparatus

1. The sweet lime peels were collected from a local juice shop and were washed under running tap water to clean them and to remove any residues.
2. The peels were ground in a mixer with distilled water to get a coarse particle slurry.
3. The round bottom flask was filled with the grinded material and the RBF was filled with distilled water up to half of the RBF capacity.
4. The temperature was maintained at 80-90°C.
5. The desired amount of oil got collected in the burette portion of the apparatus and was collected the same in small Eppendorf Tubes
6. The material in the RBF was filtered through a muslin cloth. The filtrate was concentrated by placing small amounts in petri dishes on a burner.
7. The concentrated filtrate was added and ethanol was added to it for precipitation.
8. The pectin obtained was dried in the oven and then powdered to get fine pectin.

Physical and chemical properties of garden cress seed extract

1)Phytochemical screening of ether extracted

The Garden cress seeds were subjected to ether extraction and the phytochemical screening for the seed extracts was done. The method of extraction used was Soxhlet extraction [22,23,24].

Shinoda test

Shinoda's test for flavonoids: About 0.5 of each portion was dissolved in ethanol and then the supernatant was tested. Three pieces of magnesium chips were then added to the filtrate followed by a few drops of

concentrated HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids [25, 26].

Test for tannins

About 0.5 g of the extract was taken and A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration [27].

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids [28].

Ruthenium red test

A small quantity of dried sample powder was taken and was mounted on a slide with ruthenium red solution, and was observed under the microscope [29].

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish-brown colouration of the interface indicates the presence of terpenoids [30].

Test for carbohydrates

2 ml of sample was taken in dry test tube. 2 ml of distilled water was taken in another tube as control. 2-3 drops of Molisch's reagent were added to the solution. 1 ml of conc. H2SO4 was passed along the side of the tube so that two distinct layers formed. The colour change at the junction of two layers was observed. Appearance of purple colour indicates the presence of carbohydrates [31].

2)Physicochemical test for ether and hexane GCS oil

Saponification value

1. About 2g of the substance was being examined, accurately weighed, into a 250 ml round bottom flask with a reflux condenser.
2. 25 ml of 0.5 ethanolic potassium hydroxide was added along with a little pumice powder
3. Boiled under reflux on a water bath for 30 mins.
4. 1 ml of phenolphthalein solution was added and titrated immediately with 0.5 M HCL
5. The blank was performed [32].

Refractive index:

1. The surface of the prism was cleaned first with alcohol and then with acetone using cotton and allowed to dry.
2. Using a dropper 2-3 drops of sample oil/liquid were put between the prisms and the prisms were pressed together.
3. The light was allowed to fall on the mirror.
4. The mirror was adjusted to reflect maximum light into the prism box
6. The prism box was rotated by moving the lever until the boundary b/w shaded and bright parts appeared in the field of view.

7. If a band of colours appeared in the light shade boundary. It should be made sharp by rotating the compensator.
8. The lever was adjusted so that light shade boundary passed exactly through the centre of cross wire
9. The refractive index was read directly on the scale set of readings and find the average of all the readings [33].

Iodine value:

1. 200mg of GCS oil was weighed and was placed in a dry iodometric flask.
2. Fat was dissolved in CCl4, and 25 ml of pyridine bromide solution or iodine monochloride was added, the flask was stopped and the flask was kept in dark for about 20 mins.
3. The flask was shaken from time to time.
4. 15ml of KI solution was added and the stopper & neck of the flask was rinsed with the same. 100 ml of purified water was added
5. The liberated iodine was titrated with standard Na, S2,03 (0.IN) using starch as an indicator towards the endpoint.
6. A blank was performed without sample [34].

Acid value

Standardization of KOH solution.

1. 10 ml of 0.1 N oxalic acid solution is added in a conical flask. 2-3 drops of phenolphthalein indicator are added to the solution.
2. Titrate the solution with KOH. Note the burette reading.
3. Determine the correct normality of KOH using the formula N, V, =N, V,

Determination of acid value

1. Weigh 2.5 gm of oil sample in 50ml conical flask.
2. Add 25 ml of mixture of alcohol & ether (1:1). Mix thoroughly.
3. Titrate against 0.1N KOH using phenolphthalein as the indicator [35].

3)Biological activity of GC seed oil

Antimicrobial test

1. The antibacterial activity of *Lepidium sativum* seed oil at 200 mg/mL, 500 mg/mL, and 1000 mg/mL in pure Dimethyl Sulfoxide (DMSO) was assessed towards Gram- positive strains: Staphylococcus aureus ATCG 25923 and Gram - negative strain: E. coli (Escherichia coli)
2. Suspension of the tested microorganisms was spread on the relevant solid media plates (Luria-Bertani) and incubated at 37 Â°C overnight.
3. A day later, 4-5 loops of pure colonies were transferred to sterile saline solutions in a test tube for each bacterial strain and adjusted according to the 0.5 McFarland turbidity standard (~10% cells/mL).
4. Sterile cotton was dipped into the bacterial suspension and the agar plates were streaked three times, each time turning the plate at a 60Â° angle, and finally rubbing the swab through the edge of the plate. While Ampicillin (10 pg./disc) was used as positive control for all strains
5. The same solvent (pure DMSO) applied for the dilution of the *Lepidium sativum* seed oil was used.
6. Inoculated plates with discs were placed in a 37°C incubator. Within 24 h of incubation, the results were assessed by measuring the growth inhibition zones surrounding the discs [36].

Antioxidant DPPH assay

Preparation of DPPH solution

DPPH solution was prepared by taking 7.89 mg of DPPH using a chemical balance, dissolving with 100 ml 99.5% ethanol, and finally kept in dark for 2 hr.

DPPH assay procedure

A DPPH solution of 1,000µl was added with 450µl of Tris-HCL buffer (pH 7.4) in a testing tube. And then 200 µl of testing sample solution was added and mixed quickly. The solution was kept at room temperature for 30 min. The absorbance of the solution at 517 nm was recorded. A mixed solution of ethyl acetate 1,000µl and 450µl of Tris-HCl buffer (pH 7.4) was used as the blank.

The inhibition ratio (%) was obtained from the following equation:

Inhibition ratio (%) = (A1 - A2) x 100/A1,

where A1 is the absorbance of the addition of ethanol instead of testing

sample and A2 is the absorbance of testing sample solution [36].

Anti acne activity

1. Sterile Soybean Casein digest agar petri plate was prepared and inoculated with the P Acnes culture solution by using spread plate method
2. Well, was created in the centre of the agar plate by using a sterile cork borer. After the well was created, the sample solution was inoculated in the same agar well
3. 100µl of sample solution was inoculated in the well
4. Petri plate with the inoculated sample was incubated at 35-37 degree Celsius for 4-6 days at anaerobic condition
5. After incubation, growth of P Acnes was observed and the zone of clearing or inhibition was measured [37].

Result and discussion

GCS oil extraction

Table no. 6 GCS was extracted using two solvents: ether extracted oil (EEO) and hexane extracted oil (HEO)

Extraction solvent /procedure	Hexane Soxhlet extraction	Ether Soxhlet extraction	Distilled water / Clevenger's
Yield experimental	15.8%	12%	10.89%
Yield reported [43][39][38].	12.7%	6.4%	52,2%

Therefore, the GCSO extracted with hexane with Soxhlet extraction has better yield as compared to the ether extracted GCSO

Physical and chemical properties of garden cress seed oil

The GCS oil was evaluated to check physical and chemical properties.

1)Phytochemical screening of ether extracted

Table no .7 Results for petroleum ether extracted oil

Test name	Experimental results	Reported results
Shinoda test	Flavonoids present	Flavonoids absent
Tannins test	Tannins present	Tannins absent
Alkaloids test	Alkaloids absent	Alkaloids absent
Ruthenium red test	Mucilage absent	Mucilage absent
Salkowski test	Terpenoids absent	_____
Molisch test	Carbohydrates absent	Carbohydrates absent

Shinoda test

On the addition of the reagents the colour changed to reddish pink, confirming the presence of the flavonoids. The ether extract of *Lepidium sativum* seeds was used for the phytochemical screening. Our results do not match with K. Chatoui et al [24]. The reason behind this may be the difference in extraction procedure used. Soxhlet extraction was carried out in the lab whereas the research articles have reported the use of simple maceration and drying by rotary evaporation.

Test for tannins

In this test the colour changed to brownish green to confirm the presence of the tannins. The ether extract of *Lepidium sativum* seeds was used for the phytochemical screening. Our results do not match with Rizwan and et al [22]. They have reported the absence of tannins in the ether extract but they have reported presence of tannins in alcoholic, aqueous and chloroform extract. This may be a false positive test, as tannins are in the layer between the external tegument and the aleurone layer of the seeds. Therefore, a probable reason can be the very fine grinding of GC seeds leading to rupture of the external tegument and the aleurone layer thus leading to contamination of the oil with tannins during the extraction procedure.

Test for alkaloids

There was no presence of a brown precipitate and hence we confirm the absence of alkaloids. Our result matches with the findings of Rizwan et al [22]. However, this article has reported the presence of alkaloids in chloroform, aqueous and alcoholic extracts respectively.

Ruthenium red test

There was no development of pink when the slide was seen under the microscope. This indicates the absence of mucilage in our sample. Our result matches with the findings of Rizwan et al [22]. However, they have reported the presence of carbohydrates in aqueous extract.

Test for terpenoids (Salkowski test)

There was absence of reddish-brown colouration at the interface which clearly indicates the absence of terpenoids.

Test for carbohydrates

The absence of purple colour at the junction of the two layers which indicates the absence of carbohydrates in the sample. Our result matches with the findings of Rizwan et al [22].

2) PHYSICOCHEMICAL PROPERTIES OF HEXANE AND ETHER EXTRACTED GC SEED OIL

Table no. 8

		WITH EEO	WITH HEO
Attributes	Soxhlet extracted values (Reported values)	SEV (Experimental values)	Experimental Values
Refractive index(nDt) [40].	1.47+ 0.003	1.46	1.25
Saponification value (mg KOH/g)[40].	183.23 + 0.73	5.61	16.66
Iodine value (gof I2 absorbed/100 g) [40].	122	110.8	105.84
Acid value [40]. (mg KOH/g)	0.39	1.6392	1.562

Saponification value

SAP value tells us about the average molecular weight of the large medium or small chain fatty acids present in the fat sample. Higher the SAP value more is the presence of short chain fatty acids. The values reported as per Chandra Shekar et al [40] are very high as compared to the values obtained by our team. This is due to the aging of oil as the freshly extracted oil was not used during the mentioned experiment. Due to aging the ester links in the oil break releasing the fatty acids thus leading to a greater neutralization of fatty acids by KOH and thus less KOH remains for back titration with standardized HCl solution.

Refractive index:

Garden cress seed oil extracted with ether has a value of 1.47 which agrees with the findings of Chandra Shekar et al. [40]. The refractive index of Garden cress oil extracted with hexane has a lower value than ether extracted oil. The possible reason behind this can be traced back to the findings of M.R. Norazlina et al [41]. In their findings they have compared the refractive indices of oils extracted with different solvents. They reported that hexane extracted oil has the highest yield but lesser unsaturation [41]. Edible oils have refractive indices in the range of 1.3-1.5 which signifies the use of Garden cress seed oil in jellies and other edible products [40]. This high refractive index value signifies the presence of unsaturation in the oil [42]. The refractive index value of hexane extracted GCSO is less in accordance with findings of Sachin Maske et al [43] but not in accordance with findings of Chandra Shekar et al [40].

Iodine value:

Iodine values of GCSO ether extracted and GCSO hexane extracted are 110.8 and 105.84 respectively. Iodine value is the measure of unsaturation in oil. The values are lesser than the values reported by Chandra Shekar et al as the oil extracted by our team has not been subjected to further purification. The reason behind the lesser value of hexane extracted oil is probably due to lesser unsaturation seen in the hexane extracted oil [41]. The probable reason behind the less value of iodine can be traced back to the findings of S Naz et al. Iodine value has an inverse relationship with the oil storage [44].

Acid value:

Acid value signifies about the free fatty acid in the oil sample. The acid value GCSO ether extracted and GCSO hexane extracted are 1.6392 and 1.562 (mg KOH/g) which is greater than the values obtained by Sachin maske et al [43]. Less acid value signifies the stability of oil at room temperature [45]. The reason behind this can be the increasing storage time of oil. According to the findings of Govind B Yenge and et al, the acid value is directly proportional to the storage time [46]. This can be validated by the finding of Ogbonnaya Chukwu and et al that increase in acid value leads to the increase in activity of enzyme lipase [47].

3)BIOLOGICAL ACTIVITY OF GC SEED OIL

Antimicrobial test

We tested three concentrations of our GC seed oil extracted with the help of Soxhlet extraction and the solvent used was ether. Three different concentrations were used: 1000 mg/ml, 500mg/ml, and 200mg/ml. The antimicrobial test showed the inhibition zones of diameter 7mm, 5mm and 3mm respectively.

Table no. 9 Antioxidant DPPH assay with hexane extracted oil

Conc. of hexane extracted oil	Percentage inhibition
10mg/ml	58.33 %
30mg/ml	70.88%
60 mg/ml	79.11%

Table no. 10 Antioxidant DPPH assay with ether extracted oil

Conc of ether extracted oil	Percentage inhibition]
10mg/ml	62.5%
30mg/ml	75%
60 mg/ml	90%

Antioxidant DPPH assay

DPPH solution conc. - 0.00007mg/ml

Tris HCl buffer(ph-7.4) - 0.1M

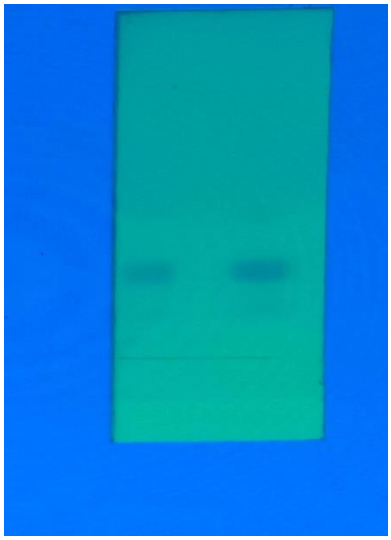
Hexane extracted oil
Conc abs
10mg/ml - 0.10
30mg/ml -. 0.07
60 mg/ml -. 0.05
Ether extracted oil
Conc. Abs
10mg/ml - 0.09
30mg/ml -. 0.06
60 mg/ml -. 0.02
Negative control: DPPH solution + ethyl acetate
Abs reading of control - 0.24
Positive control - Tris buffer + ethyl acetate BHT
Positive control reading - 0.07
The above readings show that both extracted oils have antioxidant activity associated with them. According to the find4ings of Fulwah et al 2018, the concentration used by them is 40,32,24,16,8,5 mg/ml respectively. The result obtained by them for the highest and and the lowest concentration is $50 \pm 0.7\%$ and 22.15% respectively [48]. The reason behind the differences can be the difference in the concentration of the DPPH solution used. Also, the amount of Tris buffer used by Fulwah et al 2018 is different. But the results approximately match after taking their used concentrations for different reagents into consideration.

Anti acne activity

There was no anti acne activity presented by the GCSO sample and therefore GCSO cannot be formulated into a dermal formulation having anti acne activity.

4) TLC TEST SOLVENT SYSTEM USED FOR TESTING CITRAL IN SWEET LIME OIL [49][50].

Ethyl acetate: toluene 93:7
Reported rf value for citral :0.45
Experimental rf value for citral: 0.4
Image of the tlc plate



This confirms the presence of citral in the sweet lime oil sample thus extracted from sweet lime peel

Table no. 11 EVALUATION OF JELLIES

GELLING AGENT USED TEST NAME	PECTIN	AGAR	GELATIN	CARAGENAN	CHINA GRASS
CLARITY	CLEAR	NOT CLEAR	CLEAR	CLEAR	CLEAR
COLOUR	YELLOW	WHITISH YELLOW	YELLOW	YELLOW	YELLOW
CONSISTENCY	MELTING AT ROOM TEMP	DRY VISCOUS	THICK	JELLY STRUCTURE	JELLY STRUCTURE
STICKINESS	STICKY	NOT STICKY	MORE STICKY	LESS STICKY	LESS STICKY
GRITTINESS	NOT GRITTY	NOT GRITTY	MORE GRITTY	NOT GRITTY	NOT GRITTY
Before incubation	3.61	6.80	6.30	6.90	6.80
After incubation	3.61	6.3	6.30	6.65	5.57
After 24 hours	3.6	4.16	6.30	6.65	7.12
Stability of jellies	Melting at room temperature	Dry viscous did not retain jelly structure	Thick, jelly structure retained	Jelly structure retained	Jelly structure retained

GC seed jellies VS GC seed oil jellies

1. The GC seed jellies when added in the formulation make them less children complain due to bitter taste of the seeds that comes along even if the taste masking agent is added in the jellies. A better alternative to bitter GC seeds can be the GC seed oil which eliminates the bitter taste of the GC seeds and removes a disturbing/al/awkward feeling while consuming the jellies.
2. 2)The GC seed when added to the formulation do not get homogeneously dispersed in the formulation instead, they just remain suspended. A better alternative to this can be the use of GC oil which disperses homogeneously throughout the jelly
3. Dose calculation harder in case of GC seeds but easier in case of GC seed oil

4. Swelling of seeds increases the stability of the jelly and makes the jelly retain its structure. The biggest disadvantage of GC seed oil is that it lacks the swelling property of seed thus making the formulation difficult.
5. The GC seeds during the formulation heating procedure swell even more instead of degradation. The GC seed oil on the other hand will probably degrade due to excessive heating procedure and can lead to production of unwanted byproducts
6. The amount of jelly formed in case of GC seeds is a lot more as compared to the GC seed oil jellies, thus leading to less gelling powder requirement.
7. All fatty acids flavonoids and compounds like phenyl acetonitrile, sabinene, tocopherol, cineole, limonene, thujone, delta- 5- Avena sterol, furfuranol are oil soluble and amino acids like aspartic acid, histidine, glutamic acid, serine, and tannins, glucosinolates, cardiac glycosides are not oil soluble [51, 52, 53].

Conclusion

1. We conducted phytochemical screening of the GCSO and physicochemical test consisting of Sap value, iodine value, acid value and refractive index for the same.
2. The ingredients used in the formulation of GC seed jellies such as honey, citric acid, GCS, gelling agents like pectin, gelatine, carrageenan have a synergistic effect and have the potential to help as an adjuvant therapy supplement in various diseases such as diarrhoea, diabetes, inflammatory diseases
3. GC seed and GC seed oil jellies both contain the required nutrients, heavily fortified with flavonoids, mufa and pufa etc which are responsible for antioxidant, anti-inflammatory and antianemia properties.
4. These two formulations also provide and fulfil a person's daily intake of vitamins and all essential minerals
5. However extensive research needs to be done on the stability of oil in the formulation and on the probable by products that might get formed during the heating procedure while formulating the jellies.

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