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Cultivation, Extraction and Evaluation of Antibacterial Activity of *Carthamus tinctorius* (Safflower) oil against E, *coli*

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ABSTRACT:

Carthamus tinctorius (Safflower) is a profoundly expanded, herbaceous, thorn like yearly therapeutic plant which has been cultivated commercially for vegetable and medicinal applications. The Safflower seed was cultivated in Chandrapur region of Maharashtra in month of March to June. Seed has been procured for cultivation from Agriculture Department; Maharashtra. It was planted in the soil at distance of 2x3 ft. in lines. The seeds have been collected from plant after appropriate maturing in the long stretch of May. Then it was further proceeded for oil extraction. The oil was removed by oil extractor machine which works on principle of friction and continuous pressure. The safflower seed oil is viewed as a superior oil since it contains higher measure of oleic and linoleic acid than other oil seed crops. Safflower oil has various applications in food, beautifiers, medicinal and feed industry.

Antibacterial action of safflower oil has been performed against bacterial culture of *E. Coli*. The activity was observed by well diffusion method. By this method safflower oil shows zone of inhibition against bacterial culture of *E. coli*. Study shows that Safflower oil is work as a potential antibacterial agent against *E. coli*. Additionally, Safflower oil used to treat skin diseases, bone related issues, menopause and atherosclerosis.

Keywords: Antibacterial activity, Carthamus tinctorius, E. coli.

1. INTRODUCTION-

Safflower (Carthamus tinctorius) is an oilseed crop which has been grown on a relatively small scale in parts of Rajasthan and low rainy areas of India [(1).] This plant is cultivated mainly for its seed, which is employed as edible oil and as birdseed. This crop has also grown for its flowers, coloring and flavoring foods and making dyes. It has become an increasingly important crop in some parts of the planet including Turkey and Iran thanks to the rich content and high nutritional value of its edible oil. It contains a high amount of polyunsaturated carboxylic acid linoleic acid (70%) and monounsaturated fatty acid (10%) with small amounts of octadecanoic acid [(2).] Safflower (Carthamus tinctorius L), is a famous traditional medicine which has many effects such as anticoagulant, vasodilator, antioxidant, immunosuppressant, and neuro protector. Carthamus tinctorius with an aim to seek out most active antibacterial agents of those extract with different concentrations [(3).]

Botanical Characteristic

Botanical and Morphological Characteristics Safflower is a member of the Asteraceae family which constitutes approximately 22,750 genera and more than 1620 species in the order Asterales [**(4).**] Carthamus species may originate from Southern Asia, and are annual thistle-like plants with many spines on leaves and bracts, cultivated mainly in dry, hot climate conditions. They can reach a height of 0.3 to 2.1 m and their axillary flowers grow in the leaf axils. Flowers are initially orange and later change into a red colour. The total bloom stage may last for four weeks or more. The heads with upper leaves are up to 4 by 3 cm long. The bracts are light green and have thorny tips with a thorny appendage [**(5)**]. The fruit is 6 to 8 cm long, obovate or pear-shaped, and bluntly wedge-shaped at the base with protruding long ribs. The leaves are long, rather soft, and glabrous with a thorny-serrate margin and tip. The size of the leaf varies widely from species to individual plant and usually ranges from 2.5 to 5 in width and 10 to 15 cm in length [**(6).**]

Fig 1: Cultivated safflower crop

Medicinal Applications [7] [8]

S.No.	Clinical Uses
1.	**Heart Disease:** Safflower dilates arteries by lowering blood cholesterol, reduces hypertension and increases blood flow and, hence, oxygenation of tissues. In 90% of patients it also inhibits thrombus formation and, over time, dissolves thrombi. In 83% of patients with coronary disease, blood cholesterol levels have been reduced after 6 weeks of treatment. Hence it can be used by persons suffering from cardio vascular disorders
2.	**Constipation**: A very popular Unani laxative medicine called Twarishe Qhurtum is prepared from safflower seeds
3.	**Asthma:** Safflower seeds acts as an expectorant and reduces the spasms by liquefying the tenacious sputum. Safflower, along with other herbs, has been used to treat respiratory diseases including pertussis (whooping cough) and chronic bronchitis.

4.	**Sexual Debility:** Safflower decoctions have been used successfully for treatment of male sterility and dead sperm excess disease.
5.	**Female Disorders:** A brew made from safflower foliage is said to prevent abortion and the female sterility. Laborcan be induced by a preparation of safflower, ideally along with rupture of membranes
6.	Rheumatism: safflower is successful in treatments of sciatica and thorax rheumatism. Safflower wine is recommended for 62 types of rheumatism. Safflower prescriptions have been very effective treatments for rheumatoid arthritis
7.	Chronic hepatitis (hepatitis C):EH0202 is a traditional Japanese Kampo therapy containing safflower seed extract and is used for immunostimulation. EH0202 may decrease hepatitis C virus-RNA levels in patients with high viral titers . More studies are needed to describe safflower's effect on hepatitis C.
8.	Diabetes mellitus type 2: Lipid (fat) abnormalities are commonly associated with diabetes, and complications of atherosclerotic disease are frequently associated with diabetes. Safflower oil may negatively affect glucose metabolism due to the extra intake of energy or fat, but these effects may be less pronounced than in fish oil.
9.	Hypertension(high blood pressure): Based on preliminary evidence, safflower oil may be involved in synthesis of prostaglandins, which are responsible for vascular regulation and inflammatory responses and may affect hypertension (high blood pressure). However, clinical studies have shown that safflower oil ingestion decreases or does not affect blood pressure. Due to the conflicting evidence, additional study is needed in this area
10.	Total paranteral nutrition: Parenteral nutrition requires a certain

	percentage of fats to provide full nutrition. Various sources of fats have been used, including safflower oil. Overall, clinical trials have shown safflower oil total parenteral nutrition (TPN) to be safe when used at the doses in the trials. However, more studies should be conducted to see if safflower oil is superior to other sources of TPN lipids.

Different applications of safflower in the traditional textbooks [(9) (10)]

Plant part	**Properties**
Flower, seeds	Laxative effects
Seed oil	Rheumatism and paralysis
-	Easy uptake by target tissue
-	Tissue contraction
-	Treatment of vitiligo and black spots
-	Treatment of Psoriasis
-	Treatment of mouth ulcers
-	Pain relief
Fruit, leaves	Phlegm humor (Balgham) reduction
-	Anti-poison (scorpion)
	Treatment of numb limbs
Seeds	Laxative
Seeds	Laxative
Seeds	Melancholy humor (soda) reduction
Seeds	Semen improvement

Chemical Composition of oil

More than 200 compounds have been isolated from C. tinctorius and the commonly known ones are flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids and polysaccharides. Analysis of safflower seeds showed that crude protein ranged from 14.9% to 17%, total sugar from 3.2% to 9.2% and extractable lipids from 25% to 40%.(16) Oil content of the seeds is similar to that of olive and includes linoleic acid (63%–72%), oleic acid (16%–25%) and linolenic acid (1%–6%) [(2)(11).]

- **Materials and Methods**

Materials

Fresh seed of safflower oil were used to analyze. Due to their low humidity, seeds were stored in a dark and dry place at room temperature. All chemicals and solvents used in this study were analytical reagent grade

Seed collection

The seed use in this study was collected from Department of Agricultural Maharashtra state. The collected seed was proceed for germination. In the germination process 100 seed was taken and kept in jute bag with some moisture and humidity. After interval of each day seed germination was observed and count the germinated seed. The percentage of germination was found to be above 70%.[12] [13]

Fig 2: Safflower Seeds

Seed Treatment

Rhizobium (2%), Phosphate solubilising Bacteria (2%) and Thiram (75% WP) used for protection against seed rots and damping-off caused by soil- and seed-borne fungi. Seed treatments have very effective against Alternaria, which has been carried inside the seed. Damping-off is the loss of seedlings. It is mainly caused by soil-borne micro-organisms and occasionally seed-borne Alternaria. Do not grow safflower in fields where wireworms and cutworms expected to be a problem [14] [15]

Seeding Date

Safflower seed was planted during the month of March to April. Safflower seed planted after the month of May have great risk from fall frosts, with the maturing process being arrested; resulting in

reduced yields. Later seeding also frequently results in seed filling occurring at a time when soil moisture is low. Early April seeding is not beneficial in many years as practically no germination occurs [16].

Seeding Depth

The depth for safflower was kept at 2 to 3.5 cm (0.75 to 1.5 inches) into a well-packed, moist soil because of its hulled seed, needs more moisture than a cereal grain to germinate. During seed sowing precaution was taken about seed that it should not be placed too deeply. Safflower is very sensitive to deep seeding, particularly if the soils are wet. Deep seeding extends the period during which the seedling exposed to damping off pathogens, which can lead to uneven stands [16].

Seeding Equipment

The safflower seed was sowed in the soil with help of seeding equipment like Disc drills, hoe drills and air seeders. Seed was seeded with precaution that it achieves a uniform, shallow seeding depth and place and pack the seed into moist soil. Safflower seed have about the same size as a barley seed so setting of equipment was adjusted according to size of safflower seed. Cup calibrations on most drills are the same as for barley but set as if the seeding rate were 32 to 40 kg/ha (30 to 35 lb/ac) [17].

Irrigation

Irrigation was done for safflower seed because of limited moisture prior to flowering and seed filling. one irrigation was given at the bud stage which helps to the double the yields During irrigation special precaution has been taken into consideration that excess water application avoided it results in delayed maturity and increased disease. Irrigation before the bud stage was avoided because it delay maturity by as much as 5 days and irrigation during the bud stage can delay maturity by a further 10 days. Irrigation beyond flowering was given; it increases the risk of Sclerotinia as well as Alternaria leaf blight. During irrigating safflower we kept the soil at 60 to 75% of field capacity once the crop has formed a rosette and weed control was complete. In necessary, irrigation was given for the last time in early August, before the end of flowering. This replenishes the soil profile and the crop can mature using the available soil moisture. Further irrigations was avoided because it delay maturity and increase the risk of frost damage [18].

Extraction of oil

The seeds were sieved manually in order to clean them from impurities and dust. The seed collection was done with the hydraulic equipment and manually also. After collection of seed it subjected to oil extraction process. The extraction of oil was performed by using traditional ghani. The ghani

comprises of pestle and mortar and it driven by engine or sometimes bull. The safflower seeds were placed in the mortar and movement of pestle grind the seeds to remove the oil. This whole process takes 8 to 10 hr to complete extraction from 100 kg of seed. Extracted oil was filtered and then kept it in drum to settle down the particles from oil[19][11].

Fig 3: Oil Extraction by (Ghani) and Electric Extractor

Economic Exploitation

After getting the oil from safflower seed economic cost was calculated. In this 100 kg of safflower seed was subjected for oil extraction process. From 100 kg of seeds we get the 35 kg oil and 60 kg of safflower seed cake which was given to domestic animal for improve milk production. From the estimation of this oil and seed cake gives near about 20,000 rupees behind per 100 kg of safflower seed [20].

Physical Properties of Safflower Seed

Moisture content of safflower seed was determined by using oven. In which 1 gm of seed was taken and kept at 600c and again measure the weight of seed and calculate the difference between weights. To determine the length, width, thickness, weight, 30 seeds were selected. Dimension properties were carried out by using a vernier calliper with an accuracy of ±0.01. Weight values of single seeds and one-thousand seed weight were obtained with an electronic balance with accuracy of 0.001 g. Some of determined physical properties were given in results [21].

Ash Value

Standard method was used to determine the contents of ash method. Weight accurately about 5 gm of seed. Kept a dish and take a weight of dish then it kept in a muffle furnace for 2 h at 600 °C. Then

remove sample from muffle furnace and cooled it at room temperature .Results were expressed as a percentage of the dry basis [(22).]

Antibacterial activity assays

The antibacterial activity of the safflower oil was determined by using the agar diffusion method against *E, coli*. Bacteria were inoculated in nutrient broth and incubated at 37 °C for 24 h. Then, 1% of the bacteria were added into the nutrient broth and re-incubated for 18 h. The microbial cultures (1%) were pipetted, sowed by the spread plate method and left for 20 min. Then, 4 equidistant wells were bored by sterile cork borers (Bore size=8 mm). The safflower oil were dissolved in the solvent (water) as a final concentration of 10% (w: v), and different concentration of the safflower oil was applied to the wells. Plates were incubated for bacteria at 37 °C, and the diameters of the inhibition zone (mm) were then detected after 18-24 hr [(23).]

Result & Discussion:

Physical Properties of Safflower

The safflower seed was collected from crop cultivated as per reported method. In the cultivation all parameters are considered like, seeding, seed treatment, irrigation, extraction of oil and yield of the product. From the cultivated seed some seed are selected and subjected to testing of parameters like density, refractive index, specific gravity, and viscosity were determined. The results obtained are depicted in Table 1

Table 1: Physical Properties Safflower

SN	Physical Properties	Results
1	Length (mm)	7.27
2	Width (mm)	3.5
3	Thickness (mm)	2.8
4	Density (g/ml)	0.960
5	Specific gravity	0.925
7	Viscosity (cP)	48
8	Ash Value	3.40%

From the above table it is revealed that the density is the ratio of mass per unit volume. The density of the safflower oil was found to be 0.960. Generally, specific gravity of oils increases with unsaturation levels. The different proportion of C18 polyunsaturated fatty acids could be a major

factor for the increase in the specific gravity of the oil, and it is temperature-dependent and decreases when the temperature increases. The specific gravity of safflower oil was noted 0.925. Ash value of seeds was determined by reported method and it found to be 3.40%, Viscosity was observed as 48CP.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

It is obviously that all tested bacterial species did not show visible growth. The table shows that Safflower plant extracts revealed antibacterial activity against *E, coli.* The minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) test was performed against *E. coli.* Determination of MIC was done by taking 1%, 2%, 3%, aqueous conc. of safflower seed oil for inoculation to well and it incubated for 24 hrs. Zone of inhibition was measured against the same bacterial culture result shows that 1%, concentration of safflower seed oil does not show zone of inhibition whereas 2%, concentration of AgNPs showed zone of inhibition against *Escherichia Coli.* From this it confirms that 2%, conc. was the minimum inhibitory concentration of safflower seed oil.

Then MBC safflower seed oil was determined by taking 2-4% of safflower seed oil inoculated to well and incubate for 24 hours. 2%, conc. shows the zone of inhibition after that it was kept under observation no visible bacterial growth reappear on area of zone of inhibition that meance 2%, conc. considers as Minimum bactericidal concentration. This was done by observing pre and post-incubated agar plates. In this study, The MIC and MBC of safflower seed oil against *Escherichia Coli.* Were determined and were found to be effective at 2% conc. The safflower seed oil showed MIC at concentration of 2% conc. against the culture of *Escherichia Coli* and same concentration act as Minimum Bactericidal Concentration. Conc. of safflower seed oil can be observed in Table 2

Table 2: MIC and MBC Determination of safflower oil against *E, coli*

	MIC observations				**MBC observations**			
	E,coli				***E,coli***			
Conc. of safflower seed oil	Control	1%	2%	3%	Control	2%	3%	4%
Zone of Inhibition	-	-	+	+	-	+	+	+

Positive (+): Indicating Zone of Inhibition; Negative (–): Indicating No Zone of Inhibition

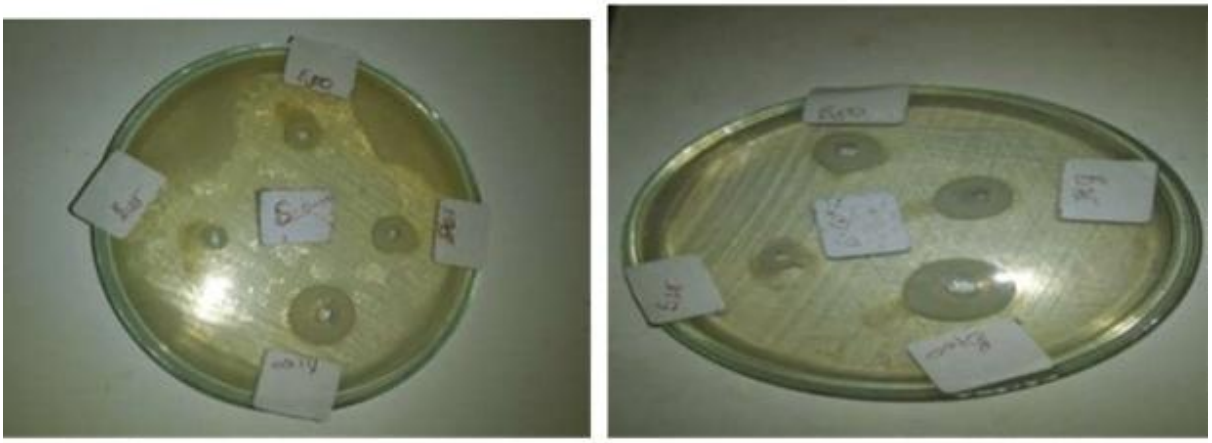

Fig: 1 safflower oil against *E, coli*

Conclusion:

Results of the present study support the traditional utilization of Safflower and it can be recommended for various medicinal purposes. The safflower seed has been cultivated successfully. The physical parameter of safflower oil was evaluated by different methods. The present study revealed that the oil extracted by ghani from the seeds of safflower (Carthamus tinctorius L.) exhibited high antibacterial effects.

Antibacterial activity of safflower seed oil was checked against the *bacteria E, coli* and it is effective against *E, coli.* From the research work it conclude that safflower seed oil shows antibacterial activity against *E, coli*

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