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Oil Production And Biological Activities For Active Compounds From *Helianthus Annuus* Seed Oil As Important Source In Biofuel And Traditional Medicine

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Abstract
In the present study, Fresh seeds of *Helianthus annuus* were collected during summer season and were dried in the sunlight for 24 hours. The dried seeds were kept in the oil machine and the oil was extracted by heat press method (at 160 °C). Around 45ml of the oil was extracted from 100 gm of seeds. About 45% seed oil was obtained from fresh seeds of sunflower. Extracted microbial oil with *Helianthus annuus* seed waste material was been used for isolation of oil. The seed waste with *Saccharomyces cerevisiae* was kept for reaction for one week and biofuel was extracted in the present work. The oil from *Helianthus annuus* seed oil contains Protein, Carbohydrate, amino acids, Terpenoids, Cholesterol, Quinones, flavonoids and Cardiac glycosides. The seed oil has shown little activity with *Candida, Enterococcus, Pseudomonas and Staphylococcus*. Less antimicrobial activity was observed with Petroleum ether or Ethanol dissolved with microbial oil (50:50).The proteinases like Trypsin and Proteinase K has shown gelatin degradation indicates the proteases making positive effect with gelatin degradation and protein degrading activity. The oil with proteinase has not shown gelatin degradation indicates the oil mix with proteins making negative effect with gelatin degradation. Caffeic acid, Chalcone, Chlorogenic acid, Neoflavonoid and Quinic acid from *Helianthus annuus* seed oil are selected as ligands. Chlorogenic acid has shown good binding activity showing good antimicrobial activity compared with Caffeic acid, Chalcone, Neoflavonoid and Quinic acid.

Keywords: *Helianthus annuus,* Seed oil, Biochemical, Antimicrobial, *in silico* analysis.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one fastest growing oilseed crops and important for commercial production in India (Bera et al., 2014). The study also shown Helianthus annus L. cv. Alestar seed waste as good biofertilizers in combination with Azosporillum and Azotobacter. Increased attention has been observed for the usage of sunflower oil seeds as renewable energy source (Ramadhas et al., 2005). Sunflower oilseed is quickly gaining attention as a feedstock crop for the production of biodiesel.

Sunflower seed mainly grown for oil that contains high levels of polyunsaturated and monounsaturated fats like palmitic, oleic, stearic, and linoleic acids, tochopherols, protein, iron, zinc, phytosterols, copper, folates, and vitamin B. It is used as edible oil that contains rich source of vegetable proteins (Adeleke et al., 2020; Nandha et al., 2014; Rauf et al., 2020). The sunflower seeds have potential usages in health and nutrition. From ancient times sunflower seeds are using as important source in food and traditional medicine. Sunflower seeds has antioxidant, anti-inflammatory, anticancer, skin-protective, antihypertensive, analgesic, hypocholesterolemic, and antibacterial activities (Pal, 2011).

Figure 1: Sunflower (*Helianthus annuus* L.) plant

Sunflower is an important oilseed crop that is native to South America that contains nutritional and medicinal value (Adeleke and Babalola, 2020). Sunflower contains phytochemicals and mineral elements such as dietary fiber, vitamins, tocopherols, phenolic acids, a-tocopherol, glutathione reductase, chlorogenic acid, manganese, phytosterols, triterpene glycosides, flavonoids, carotenoids, peptides, caffeic acid, alkaloids, tannins, and saponins. These phytocompounds contribute to their functional, medicinal and nutraceutical developments. Sunflower is cultivated all around the world to meet various nutritional, agricultural, medicinal and industrial needs. Sunflower oilcake has very short self-life that contains methionine, low lysine and 40% of protein like soy protein. The oil cake also contains proteins, oil, carbohydrates and fiber.

Figure 2: Sunflower Seed oil

Figure 3: Sunflower Seed cake as substrate

New molecular modeling and drug designing approaches are driven by rapidly improving computational platforms for conducting discovery of new mechanism- or structure-based drugs (Talele et al., 2010). Future research endeavors optimization and improvement of culture conditions, genetic engineering for producing species, complete understanding of production mechanisms, and effective techniques in biofuel production (Lee et al., 2018).

MATERIAL AND METHODS

Seed Collection

Fresh seeds of *Helianthus annuus* belong to herbaceous plant of the aster family (Asteraceae) was accumulated locally from Bilaspur region during summer season.

Biofuel Extraction

The seeds of *Helianthus annuus* were air-dried and was used for oil extraction. Oil extraction machine (at 160° C) was used for the extraction process.

Microbial Biofuel Extraction

The substrate obtained in the oil extraction was mixed with the yeast and remained for one week. The substrate + yeast sample was kept for oil extraction along with ethanol. The oil obtained was tested again with flaming.

Figure 4: Microbial substrate of *Helianthus annuus* seeds selection for oil extraction

Biochemical analysis

The extracted *Helianthus annuus* oil was tested for the biochemical activities to understand about the chemical components present in the oil.

1. Protein

To 2 ml of the extract, 2 ml of Biuret reagent is to be added. An appearance of violet color ring indicates the presence of protein.

2. Carbohydrate

To 2 ml of extract add 2 drops of Molisch's reagent and mix the solution. Nearly 2 ml of Conc. H_2SO_4 is to be added drop by drop from the sides of the test tube. A reddish violet color ring appearance at the junction of two layers immediately indicates the presence of carbohydrates.

3. Phenols

About 2 ml of extract, 3ml of ethanol and a pinch of $FeCl_3$ is to be added. The formation of greenish yellow color solution indicates the presence of phenols.

4. Terpenoids
To 2 ml of extract add 2 ml of chloroform and 3 ml of Conc. H_2SO_4. Formation of a monolayer of reddish brown coloration of an interface shows a positive result for the terpenoids.

5. Steroids
To 2 ml of acetic anhydride, 0.5 ml of extract and 2 ml of H_2SO_4 is to be added. The color changed from green or violet to blue indicates the presence of steroids.

6. Cholesterol
To 2 ml of chloroform taken in a dry test tube and add 2 ml of the extract. About 10 drops of acetic anhydride and 2 to 3 drops of Conc. H_2SO_4 are to be added to the solution. A change from red rose color solution to blue green color solution indicates the presence of cholesterol.

7. Quinones
To 2 ml of extract, 3 ml of Conc. HCl is to be added. Formation of green color solution indicates the presence of quinones.

8. Flavonoids
To 5 ml of dilute ammonia solution, a few drops of Conc. H_2SO_4 are to be added. A yellow colored solution confirms the presence of flavonoids and will disappears on long standing.

9. Amino acid
To 2 ml of the extract, 2 ml of Ninhydrin reagent is to be added and keep the solution in hot water bath for 15 minutes. The formation of purple color indicates the presence of aminoacids in the sample.

10. Cardiac Glycosides
To 5 ml of extract add 2 ml of glacial acetic acid contain one drop of ferric chloride solution is to be added. The solution was underlayed with 1ml of Conc. H_2SO_4. A brown colored ring of the edge indicates a deoxysugar is a feature of cardenolides. A violet ring may appear under the brown ring indicates an acetic acid layer and a greenish ring might form just progressively throughout thin layer.

X-Ray Film Analysis for Protein Inhibition Activity

Activity for protease inhibitor against proteases (proteinase K and trypsin) was assayed. Approximately 10 µl of protease inhibitor (*Helianthus annuus* oil) was mixed with 10 µl of protease (0.5 mg/ml) and was spotted onto a stripe of the X-ray film. 10µl of protease was mixed with 10µl of 0.lM (pH 7.0) phosphate buffer as the control and was spotted on to the X-ray film. The above inhibitor, protein and buffer mixtures were incubated of X-ray film at 37°C for 10 minutes. After 10 minutes, the film was washed under tap water gently without touching other objects for the zone of gelatin hydrolysis. The formation of clear zone as indicator of protease activity which may be due to the hydrolysis of gelatin on x-ray film.

Antimicrobial Activity

Microorganisms
Microbes from MTCC (Microbial Type Culture Collection) have been used in the present study. Various bacteria used in the present research work are *Staphylococcus aureus* (MTCC 737) and *Enterococcus faecalis* (*MTCC* 439) belongs to gram positive bacteria and *Pseudomonas aeruginosa* MTCC 424 belong to gram negative bacteria. Fungi used in the work are *Candida albicans MTCC* 227.

Antimicrobial activity using Zone method

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai., India) at 37°C for 24 hours and fungi in Sabourand Dextrose Media (Himedia Pvt. Ltd., Mumbai., India) at 250C for 72 hours, and were

maintained on nutrient agar slants at -200C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth for overnight. The overnight broth cultures were sub cultured in fresh nutrient broth and grown for 3 hours to obtain log phase culture. The agar plates were prepared by pour plate method using Muller- Hinton agar (MHA) medium for bacteria and Sabourand Dextrose agar (SDA) Media for fungi. The sterile MHA/SDA medium cooled to 45°C and mixed thoroughly with 1ml of growth culture of concerned test organism ($1x10^8$ cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 8 mm size were made with sterile borer and test extracts were added. The MHA plates were incubated at 370C for 24 hrs for bacteria. The SDA plates were incubated at 25°C for 72 hrs for fungi. The diameter of zones of inhibition was measured in mm using HiMedia zone reader.

Docking And Virtual Screening

iGEMDOCK v2.1 are the free software that has good Protein-Ligand activity. The minimum energy obtained during docking process will be the good ligand. Lower the energy higher will be the stable and effective molecule (Bugata and Kaladhar, 2014).

RESULTS AND DISCUSSION

Fresh seeds of *Helianthus annuus* were collected during summer season and were dried in the sunlight for 24 hours. The dried seeds were kept in the seeds to oil machine and the oil was extracted by heat press method (at 160 °C). Around 45ml of the oil was extracted from 100 gm of seeds. About 45% seed oil was obtained from fresh seeds of sunflower.

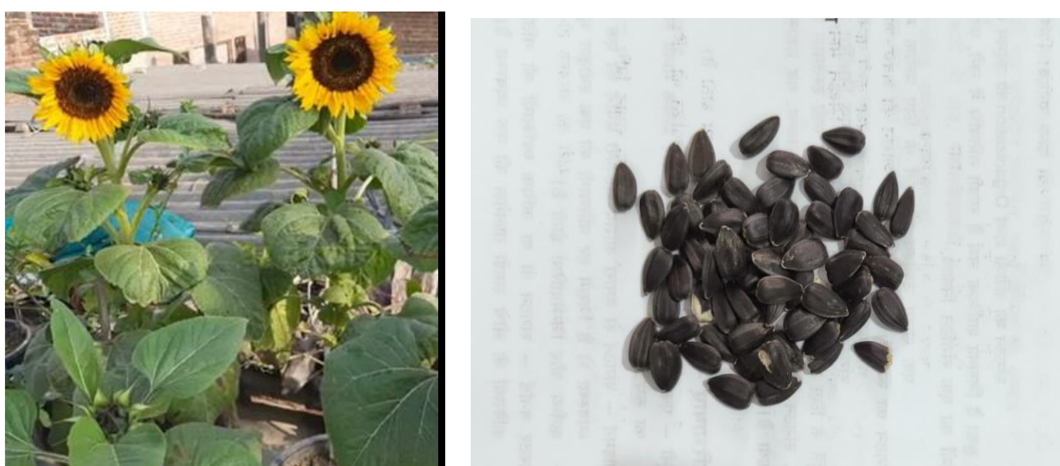

Figure 5: Plant and Seeds of *Helianthus annuus*

%oil extracted= amount of oil in ml/ 100 gm of seed X100= 45/100 X100= 45%

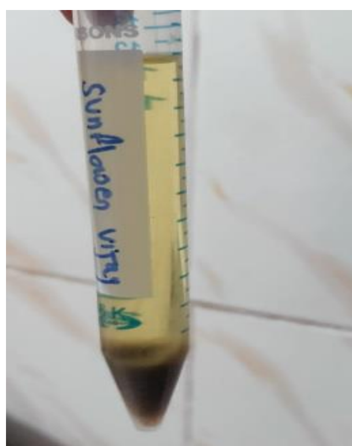

Figure 6: *Helianthus annuus* Seed oil

Figure 6 and 7 shows the extracted oil was burned in the sand pot and the oil is burned for about 10 minutes per 10ml of oil (yellow color).

Figure 7: Sand pot used for flame testing

Extracted microbial oil with *Helianthus annuus* seed waste material was been used for isolation of oil. The seed waste with *Saccharomyces cerevisiae* was kept for reaction for one week and was shown in Figure 8.

Figure 8: *Helianthus annuus* Seed waste substrate mixed with *Saccharomyces cerevisiae*

Ethanol with substrate and *Saccharomyces cerevisiae* were used for extraction of oil after one week and was tested in the sand pot for burning. The extract was observed with good orange color flame (Figure 9).

Figure 9: Microbial seed oil and Sand pot used for flame testing

Biochemical analysis

The extracted *Helianthus annuus* oil was tested for the biochemical activities to understand about the chemical components present in the oil.

1. Protein

To 2 ml of the extract, 2 ml of Biuret reagent is to be added. A violet color ring was formed that indicates the presence of protein.

2. Carbohydrate

To 2 ml of extract add 2 drops of Molisch's reagent and mix the solution. Nearly 2 ml of Conc. H_2SO_4 is to be added drop by drop from the sides of the test tube. A reddish violet color ring appeared at the junction of two layers immediately indicates the presence of carbohydrates.

3. Amino acid

To 2 ml of the extract, 2 ml of Ninhydrin reagent is to be added and keep the solution in hot water bath for 15 minutes. A purple color is formed that indicates the presence of aminoacids in the sample.

4. Phenols

About 2 ml of extract, 3ml of ethanol and a pinch of FeCl3 is to be added. The non-appearance of greenish yellow color solution indicates the absence of phenols.

5. Terpenoids

To 2 ml of extract add 2 ml of chloroform and 3 ml of Conc. H_2SO_4. Formation of a monolayer of reddish brown coloration of an interface shows a positive result for the terpenoids.

6. Steroids

To 2 ml of acetic anhydride, 0.5 ml of extract and 2 ml of H2SO4 is to be added. The color was not changed from green or violet to blue indicates the absence of steroids.

7. Cholesterol

To 2 ml of chloroform taken in a dry test tube and add 2 ml of the extract. About 10 drops of acetic anhydride and 2 to 3 drops of Conc. H2SO4 are to be added to the solution. A change from red rose color solution to blue green color solution indicates the presence of cholesterol.

8. Quinones

To 2 ml of extract, 3 ml of Conc. HCl is to be added. Formation of green color solution indicates the presence of quinones.

9. Flavonoids

To 5 ml of dilute ammonia solution, a few drops of Conc. H2SO4 are to be added. A yellow colored solution appeared that confirms the presence of flavonoids.

10.Cardiac Glycosides

To 5 ml of extract add 2 ml of glacial acetic acid contain one drop of ferric chloride solution is to be added. The solution was underlayed with 1ml of Conc. H2SO4. A brown colored ring of the edge formed indicates a deoxysugar is a feature of cardenolides. A violet ring appeared under the brown ring indicates an acetic acid layer and a greenish ring was formed just progressively throughout thin layer.

Table 1: Biochemical analysis of *Helianthus annuus* seed oil

Metabolite	Result
Protein	++
Carbohydrates	++
Amino acid	++
Phenol	-
Terpenoids	++
Steroids	-
Cholesterol	+++
Quinones	+++
Flavonoids	+
Cardiac glycosides	++

Note: +++ more positive confirmed; ++Average confirmation; +moderate confirmation; -negative confirmation

The oil from *Helianthus annuus* seed oil contains Protein, Carbohydrate, amino acids, Terpenoids, Cholesterol, Quinones, flavonoids and Cardiac glycosides (Table 1).

Antimicrobial activity using Zone method

Table 2 has shown the antimicrobial activity of the *Helianthus annuus* seed oil, microbial oil extracts and Gentamycin. The seed oil has shown little activity with Candida, Enterococcus, Pseudomonas and Staphylococcus. Less antimicrobial activity was observed with Petroleum ether or Ethanol dissolved with microbial oil (50:50).

Table 2: Antimicrobial activity *Helianthus annuus seed oil*

Sample	**Zone of inhibition (in mm) along with zone size of 8mm**			
	Candida	**Enterococcus**	**Pseudomonas**	**Staphylococcus**
Antibiotic (Gentamycin eye drops)	10	16	16	13
Seed oil	9	9	10	10
Petroleum ether mix with microbial oil (50:50)	9	9	9	9
Ethanol dissolved with microbial oil (50:50)	9	9	9	9

X-RAY FILM ANALYSIS FOR PROTEIN INHIBITION ACTIVITY

Table 3 and Figure 10 has shown the gelatin hydrolysis test for *Helianthus annuus* seed oil. Trypsin and proteinase K has shown protein degradation. The formation of clear zone as indicator of protease activity which may be due to the hydrolysis of gelatin on x-ray film. The oil was not formed zone for gelatin degradation. The proteinases like Trypsin and Proteinase K have shown gelatin degradation indicates the proteases making positive effect with gelatin degradation and protein degrading activity. The oil with proteinase has not shown gelatin degradation indicates the oil mix with proteins making negative effect with gelatin degradation.

Table 3: Gelatin degradation *Helianthus annuus* seed oil

Sample	Blank	Trypsin	Proteinase K
Buffer	-	+++	+++
Oil	-	+	+

Figure 10: Gelatin degradation activity of *Helianthus annuus* seed oil

Docking

The oil extract collected from plants *Helianthus annuus* has been searched further compounds based on previous research based on the search result, the selected compound has been absorbed as Caffeic acid, Chalcone, Chlorogenic acid, Neoflavonoid and Quinic acid.

Few *Helianthus annuus* seed compounds has been design as 3D molecule in .mol format using chemSketch software (Figure 11).

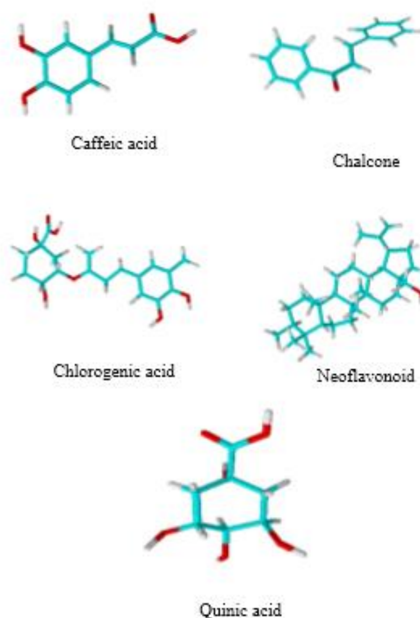

Figure 11: Selected ligands from *Helianthus annuus* seed oil

Table 4 shows the QSAR (Quantitative structure-activity relationship) properties shows no partial charges showing molecules as stable. Quantitative structure-activity relationship (QSAR) studies are unquestionably of great importance in modern chemistry and biochemistry. The *surface area* of a solid object is a measure of the total area that the *surface of* the object occupies. if temperature and pressure are constant, the number of particles is proportional to the volume. If the hydration energy is greater than the lattice energy, then the enthalpy of solution is negative (heat is released), otherwise it is positive (heat is absorbed). A negative logP value indicates preferential solubility in water and a positive value indicates an affinity for octanol. *Refractive* index is the ratio of the velocity of light of a specified wavelength in the air to its velocity in the examined substance. As polarizability increases, the dispersion forces also become stronger. Thus, molecules attract one another more strongly and melting and boiling points of covalent substances increase with larger molecular mass. Polarizability also affects dispersion forces through the molecular shape of the affected molecules. Modern mass spectrometers easily distinguish (resolve) ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound.

Table 4: QSAR properties of the ligands of *Helianthus annuus* seed oil

Ligand	Partial charges (e)	Surface Aera (A2)	Surface Area (Grid)(A2)	Volume (A3)	Hydration Energy Kcal/Mol	Log P	Refractivity (A3)	Polarizability (A3)	Mass (amu)
Caffeic acid	0.00e	334.85	379.14	579.41	18.96	0.66	50.41	17.74	180.16
Chalcone	0.00e	369.41	434.93	685.84	-3.44	3.09	75.06	25.49	208.26
Chlorogenic acid	0.00e	504.56	593.53	1007.05	-24.26	-1.90	94.55	36.03	368.38
Neoflavonoid	0.00e	313.81	414.63	666.21	-4.95	1.02	73.29	25.55	224.26
Quinic acid	0.00e	296.01	355.14	543.46	-19.34	-1.86	39.70	16.12	192.17

The receptor related to disease causing microbes with PDB ids (organism name) has been retrieve and selected as receptor (Figure 12).

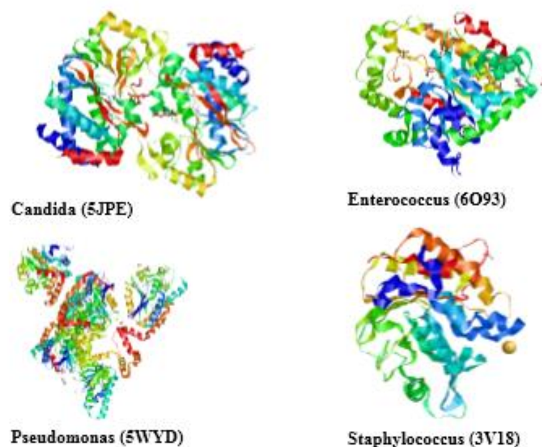

Figure 12: Selected receptors in the present study

The ligands and the receptor has been selected in the present experimentation to understand about molecular docking process

The docking result in the present experiment in Table 5. Caffeic acid, Chalcone, Chlorogenic acid, Neoflavonoid and Quinic acid from *Helianthus annuus seed oil* are selected as ligands. Chlorogenic acid has shown good binding activity showing good antimicrobial activity compared with Caffeic acid, Chalcone, Neoflavonoid and Quinic acid.

Table 5: Docking results for *Helianthus annuus seed oil* compounds

COMPOUND NAME	*Candida*	*Enterococcus*	*Pseudomonas*	*Staphylococcus*
Caffeic acid	-100.73	-61.7	-68	-74.52
Chalcones	-87.72	-72.1	-73.17	-78.8
Chlorogenic acid	**-127**	**-103.9**	**-105.48**	**-114.3**
Neoflavonoids	-94.4	-77.25	-71.26	-81.7
Quinic acid	-101	-67.4	-69.15	-79.2

The antioxidant and antimicrobial activity of methanolic extract of seeds from *Helianthus annuus* has been evaluated in previous studies (Subashini and Rakshitha, 2012; Islam et al., 2016; Guo et al., 2017). The seed extract of *Helianthus annuus* had shown less sensitivity to *Bacillus subtilis*, moderate sensitivity to *Staphylococcus aureus* and *Vibrio cholera* and high sensitivity to *Salmonella typhi*, *Rhizopus stolonifer* and *Aspergillus fumigates.* The present studies on *Helianthus annuus* seed oil has shown little activity with *Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Bio-fuels derived from plant-based resources are assumed with great importance in the present context (Dhyani et al 2015). A proper use of medicinal plant seeds and the oils requires accurate scientific information for understanding of their chemical constituents for health and hygiene.

CONCLUSION

In the present study, fresh seeds of *Helianthus annuus* were collected during summer season and were dried in the sunlight for 24 hours. The dried seeds were kept in the oil machine and the oil was extracted by heat press method (at 160 °C). About 45% seed oil was obtained from fresh seeds of sunflower. The seed waste with *Saccharomyces cerevisiae* was kept for reaction for one week and biofuel was extracted in the present work. The seed oil has shown good biochemical profiling and good antimicrobial activity.

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CONFLICTS OF INTEREST:

There is no known conflict of interest associated with the publication.

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