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Physicochemical and Anti-Microbial Properties of Sunflower (HELIANTHUS ANNUUS L.) Seed Oil

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ABSTRACT

Oil from the seeds of sunflower (Helianthus annuus L.) was extracted with n-hexane, and was evaluated for free fatty acid value, FFA (0.042%), acid value, AV (0.095mgKOH/g), ester value, EV (182.138mgKOH/g), saponification value, SV (182.233mgKOH/g), iodine value, IV (119.921mgI_2/100g), peroxide value, PV (6.322mgO_2/kg) and specific gravity, SG (0.915). The anti-microbial properties of the oil on different pathogenic organisms were evaluated.

Keywords: *sunflower seed oil, characterization, anti-microbial properties*

1. INTRODUCTION

Sunflowers are botanically classified as *Helianthus annuus*. They are a large plant and are grown throughout the world because of their relatively short growing season. Domesticated sunflowers typically have a single stalk topped by a large flower. This is significantly different from the smaller, multiply branched wild sunflower. During the growing season, the individual flowers are each pollinated, seed development then begins moving from the outer rim of the flower toward the centre. It generally takes 30 days after the last flower is pollinated for the plant to mature.

Oil can be defined as any greasy substance that is liquid at room temperature and insoluble in water [1]. Oil are heterogeneous collection of biochemical substances which have in common the property of being soluble in most organic polar solvents (chloroform, benzene, diethylether e.t.c.) and insoluble in water [2]. The dietary roles of edible oils and fats are highly recognized. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have recommended an average daily intake of 55 g-fat per capita to compliment the requirement for energy [3] and a 20-30% conversion rate for fat to energy to ensure good health[4]. Vegetable oils and fats have wide application in foods where they are used in frying, salad dressing, shortening of pasty, margarine, cooking and ice cream manufacture. Sunflowers are used to make oil, meal and confectionary products. Oil and meal are processed from the same sunflower seed varieties, the conventional sunflower oil (high linoleic) is used for home cooking oil and margarine and for industrial use (paint, etc). The high oleic sunflower oil is used for cosmetics, gasoline blend and other purposes. Edible oils extracted from plant sources are important in foods and in various other industries (e. g. cosmetics, pharmaceuticals, lubricants). They are key components of the diet and also provide characteristic flavours and textures to foods. The chemical and physical properties of edible oils depend primarily on composition (and hence on biological origin) and temperature.

This research work looked into characterisation of the sunflower seed oil and it also explored also the antimicrobial properties of the oil to minimise the overuse of antibiotics in the treatment of infectious diseases, and the appearance of `multi-drug resistant bacterial strains (resistant to two or more antibiotics) with the view to exploit its ethno-medicinal properties.

2. MATERIALS AND METHODS

Sunflower Seed

The sunflower seed used was obtained from Institute of Agricultural Research, Ahmadu Bello University- Zaria with herbarium voucher number 301.

Extraction of the oil

Dried seeds were dehusked and milled, the oil was extracted from the resulting powder by adopting the method described by A.O.A.C [5] which entailed using Soxhlet apparatus to extract the oil with n-hexane at 60-65^{o}C. The solvent was recovered from the extract by distillation and the remnant solvent was then driven off by placing the oil-solvent mixture in a water-bath leaving behind the purified oil.

Determination of the Physicochemical Properties of the Oil

A.O.A.C. [5] standard methods were used to determine the physical and chemical properties of the oil, which includes the Free Fatty Acid value (FFA), Acid Value (AV), Ester Value (EV), Saponification Value (SV), Iodine Value (IV), Peroxide Value (PV) and Specific Gravity (SG). All tests were performed in triplicate.

Determination of the Antimicrobial Properties of the Oil Tested Microorganisms

Antimicrobial activity of oil of sunflower seed was investigated against four bacterial isolates and one fungal isolate, which were obtained from Ahmadu Bello Teaching Hospital Microbiology laboratory. The bacteria used for the research work include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and the fungi used for the research work was *Candida albicans.* The tested microorganisms were cultured on Nutrient agar (for bacteria at 37^0c for 24 h) for the bacteria isolates and on Potatoes Dextrose Agar for the fungal isolates (for fungus at 30^0C for 48-72 h).

Inoculumn preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37^0C for 4 h. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1.2×10^8 colony-forming units per milliliter (cfu/ml). The grown suspension was used for further testing.

Antimicrobial bioassay

The antimicrobial activities of the extracts were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards [6, 7]. Nutrient Agar and Potato Dextrose agar was used for the antimicrobial activity test. Under aseptic conditions in the biosafety chamber, 15ml of Nutrient agar medium was dispensed into pre-sterilized Petri dishes to yield a uniform depth of 4 mm and inoculated by the bacterial and fungal culture, respectively. The sterile discs (diameter 6mm) were impregnated with different concentration (0.2ml, 0.4ml, 0.6ml) of oil of sunflower seed extract and dried for 10-15 minutes. The dried discs were placed on nutrient agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. Streptomycin of (40mg/ml) was used as positive control. The discs were spaced far enough to avoid reflections wave from the edges of the petri dishes and overlapping rings of inhibition. Finally, the petri dishes were incubated for 18 to 24 hours at 37^0C for bacteria and 28^0C for 48 to 72 hours for fungus. The diameter of zone of inhibition (mean of triplicate) as indicated by clear area which was devoid of growth of microbes was measured and recorded.

Determination of Activity Index

The activity index [7] of the crude plant extract was calculated as:

$$\text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

3. RESULTS AND DISCUSSION

Table 1 present the physicochemical properties of sunflower seed oil. The oil extracted from the sunflower seed is yellowish in color. It had a specific gravity of 0.825 which showed that it is less dense than water. The iodine value is 119.921mgI_2 /100g and oils are classified into drying, semi drying and non- drying according to their iodine values. Since the iodine value of sunflower seed oil is higher than 100 it could be classified as semi-drying or drying oil. The high iodine value indicates that the oil has a high content of unsaturated fatty acids which is evident in the acid and free fatty acid values of 0.095mgKOH/g and 0.042% respectively.

The saponification value of the sunflower oil was (182.233mgKOH/g).This was lower than the values for some common oils like palm oil (196-205mgKOH/g), coconut oil (253mgKOH/g) and palm kernel oil (247mgKOH/g) [8]. However, this saponification value fall just below the range expected of some edible oils reported by [9]. The low saponification value is an indication that the oil may not be suitable for soap making, oil-based ice-cream and shampoos.

Table 1: Physicochemical properties Sunflower Seed Oil

Parameter	Concentration
Free Fatty Acid	0.042%
Acid Value	0.953mgKOH/g
Ester Value	182.138mgKOH/g
Saponification Value	182.233mgKOH/g
Iodine Value	119.921mgI_2/100g
Peroxide Value	6.322mgO_2/kg
Specific Gravity	0.915 at 25^oC

The antimicrobial activity of oil of sunflower seed extract against the tested microorganisms examined in this study were assessed by the presence or absence of zone of inhibition and zone diameter was measured and recorded as shown in table 2 to 6. Similarly, activity index of the extract was also determined as shown in table 7.

Table 2: Candida Albicans

Number of replication	0.2ml	0.4ml	0.6ml
1	9mm	11mm	11mm
2	7mm	8mm	9mm
3	7mm	8mm	9mm

Zone of inhibition of standard antibiotic used streptomycin 40mg/ml) =28mm

Table 3: Pseudomonas Auregenosa

Number of replication	0.2ml	0.4ml	0.6ml
1	R	8mm	8mm
2	7mm	7mm	9mm
3	8mm	8mm	9mm

Zone of inhibition of standard antibiotic used (streptomycin 40mg/ml) *=32mm*

Table 4: Bacillus Subtilus

Number of replication	0.2ml	0.4ml	0.6ml
1	R	R	R
2	R	8mm	8mm
3	8mm	9mm	9mm

Zone of inhibition of standard antibiotic used (streptomycin 40mg/ml) =30

Table 5: Staphylococcus Aureus

Number of replication	0.2ml	0.4ml	0.6ml
1	7mm	7mm	8mm
2	11mm	10mm	9mm
3	7mm	8mm	8mm

Zone of inhibition of standard antibiotic used (streptomycin 40mg/ml) =28

Table 6: Escherichia Coli

Number of replication	0.2ml	0.4ml	0.6ml
1	7mm	8mm	9mm
2	7mm	8mm	7mm
3	7mm	8mm	9mm

Zone of inhibition of standard antibiotic used (streptomycin 40mg/ml) =20

Table 7: Antibacterial Activity Index of Sunflower Seed Oil

Isolates	Extract conc 0.2ml			Extract conc=0.4ml			Extract conc=0.6ml		
	ZI		I	ZI		AI	MZI	S	AI
C albicans	3mm	8mm	.821	7mm	8mm	0.964	2 9mm	2 8mm	1.036
P auregenosa	5mm	2mm	.462	3mm	2mm	0.719	2 6mm	3 2mm	0.813
B subtilis	mm	0mm	.267	7mm	2mm	0.531	1 7mm	3 2mm	0.531
S aureus	**5mm**	**8mm**	**.893**	**5mm**	**8mm**	**0.893**	**2 5mm**	**2 8mm**	**0.893**
E coli	1mm	0mm	.050	4mm	0mm	1.200	2 5mm	2 0mm	1.250

MZI=Mean Zone of Inhibition. S=Standard concentration used. AI=Activity Index

The results obtained from the antimicrobial study is interesting looking at the fact that the oil from sunflower seed was effective on some microorganisms such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Candida albicans* which are commonly involved in urinary tract infection [10] and diarrhea diseases which is one of the principal cause of death in infants[11]. *Bacillus subtillus* had the least mean zone of inhibition of 8m. Despite the fact that the zone of inhibition of the commercial antibiotic use for this research (streptomycin 40mg/ml) is higher than the zone of inhibition of oil sunflower seed, the mean zone of inhibition observed with oil of sunflower seed (at low concentration of 0.2, 0.4, 0.6ml) of up to 23mm against C albicans, 25mm against S aureus, 15mm with P aurenosa justify that oil of sunflower seed could be used as an antimicrobial agent. The activity index of the extract when compared with the concentration of the commercial antibiotic used further validate the possibilities of using the oil as a therapeutic agent.

4. CONCLUSION AND RECOMMENDATIONS

The high iodine value portrays that it is rich in unsaturated fatty acid which implies that it will have short oxidative storage stability because according to Perkins [13] the oxidative and chemical changes in oils during storage are characterized by increase in FFA content and a decrease in the total unsaturation of oils (Perkins 1992). Free Fatty Acid values was lower for the oil as compared to the value recommended by FAO/WHO [4] which may be attributed to the variation in variety, and climatic conditions which is evident in the iodine value. The slightly low saponification value in oil could be attributed to the low FFA content. The acid and peroxide values were within the FAO/WHO standard for edible vegetable oils. The specific gravity shows that the oil is less dense than water.

The antimicrobial activity indicates that despite the high mean zone of inhibition of 15mm, 21mm, 23mm, 25mm on *P auregenosa, E coli, C albicans , S aureus* respectively there is the need to further conduct *in vivo* studies with the extracts so as to confirm the present *in vitro* findings as the diameter of the zone of inhibition is not only affected by sensitivity of the microorganisms alone but also the concentration of the extract on the discs is very important and this is why *in vivo* findings such as an evaluation of the toxicity of the oil to humans at different concentration is very essential in the use of the oil for treatment and also this study paves the way for further attention and research to identify the active compounds responsible for the oil biological activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect.

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