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RESEARCH ARTICLE

GC-MS STUDIES AND ANTIMICROBIAL ACTIVITY OF SUDANESE *CAPSICUM ANNUM* FIXED OIL.

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Abstract

The present study was designed to investigate the chemical constituents of *Capsicum annum* seed oil and to evaluate its potential antimicrobial activity. 27 components were detected by GC-MS analysis. Major constituents are: 9,12-octadecadienoic acid(56.57%) , , hexadecanoic acid(17.60%), 9-octadecenoic acid (5.62%) , methyl stearate(5.50%) and stigmasterol(2.37%) . Butylated hydroxytoluene, a potent antioxidant, was detected as a minor constituent(0.22%). The antibacterial activity of the oil was evaluated via cup plate agar diffusion assay against six standard human pathogens(Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative : *Escherichia coli* and *Pseudomonasa aeruginos*a and the fungi *Candida albicans* and *Aspergillus niger*) . The oil showed different antimicrobial responses against test organisms. It was partially active against the fungus *Candida albicans* but significant activity against *Staphylococcus aureus* was observed.

Introduction:-

The genus *Capsicum* comprise all the varied forms of fleshy-fruited peppers grown as herbaceous annuals - the red, green, and yellow pepper which are rich in vitamins A and C. It includes paprika, chili pepper, red pepper (cayenne), and bell peppers[1].As paprika plants tolerate nearly every climate, the fruits are produced all over the world. A fairly warm climate is, however, necessary for a strong aroma(Andrews, 1984 ; McLeod *et.al.*,1982) **.**

Capsicum annuum is a species native to southern North America and northern South America. This species is the most common and extensively cultivated of the five domesticated capsicums. The species encompasses a wide variety of shapes and sizes of peppers, both mild and hot, ranging from bell peppers to chili peppers(McLeod *et.al.*,1982; Minguez *et.al.*1994 ;Hayman and Kam ,2008). Cultivars are descended from the wild American bird pepper still found in warmer regions of the Americas(Francis,2013 ; Zhi-Yun,2005) . In the past some woody forms of this species have been called ***C. frutescens***, but the features that were used to distinguish those forms appear in

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many populations of *C. annuum* and there is no consistently recognizable *C. frutescens* species . Although the species name *annuum* means "annual" (from the Latin *annus* "year"), the plant is not an annual and in the absence of winter frosts can survive several seasons and grow into a large perennial shrub(Alice , 2004).
The parts used are berry fruits. Removal of seeds and veins results in a less pungent and more brightly coloured product. The pungent constituents found in *Capsicum annum* are the capsaicinoids, present only in the fruit of the plant in small amounts, as low as 0.001 to 0.005% in "mild" and 0.1% in "hot" cultivars(Andrews, 1984 ; McLeod et.al.,1982).

Hot peppers, used as relishes, pickled or ground into a fine powder for use as spices, derive their pungency from the compound : capsaicin (8-methyl-N-vanillyl-6-enamide), a substance characterized by acrid and burning taste, that is located in the internal partitions of the fruit. Capsaicin stimulates gastric secretions and, if used in excess, causes inflammation. Most of the capsaicin in a pepper is found in the interior ribs that divide the chambers of the fruit, and to which the seeds are attached(Alice , 2004**)**.

Hot peppers are used in medicine as well as food in Africa and other places around the world. *C. annuum* is used traditionally for gout, dyspepsia accompanied by flatulence, paralysis etc. Its most valuable application appears however to be in *cynanche maligna* (acute diphtheria) and *scarlatina maligna* (malignant scarlet fever) used either as a gargle or administered internally.

Alicia *et.al*.(2004) characterized and quantified some constituents of *C. annum* at four maturity stages(Immature green,green ,immature red and red). Individual hydroxycinnamic acids ,flavonoids,vitamin C and individual; carotenoids were characterized and quantified. Twenty three flavonoids and 5 hydroxycinnamic derivatives were identified from pericarp by HPLC-diode array detection-electrospray ionization-mass spectrometry.

Hydroxycinnamic acid derivatives ,C-glycosides of quercetin, leteolin and chrysoeriol and a large number of C-glycosyl flavones have been characterized. Difference in the individual and total phenolic content was observed between different mature stages(Alicia *et.al*.(2004).

Aneta *et.al*.(2011) determined the content and chemical composition of capsaicinoids. Capsaicinoids were extracted with hexane and analyzed by GC/MS . The alkaloids : capsaicin,dihydrocapsaicin,nonivamide were detected as major constituents.

The carotenoid composition of some types of *Capsicum annum* have been evaluated by Keiko *et.al*. . The same authors discussed the ratio of β-carotene to capsanthin. Highest values of total carotenoids content and capsanthin were recorded for two varieties(Keiko *et.al*,.2007). Rosa *et.al*. (2013) claimed that antioxidant systems from *Capsicum annum* are involved in response to temperature changes in ripe fruits. In brain- *in vitro* studies , Oboh *et.al*.(2007) demonstrated the ability of aqueous extracts of *Capsicum annum* to inhibit Fe^{2+} - induced lipid peroxidation in rat brain.

Four new cyclic diterpene glycosides together with 12 known compounds were isolated from *Capsicum annum* fruits. Structures of the new isolates were deduced on the basis of their spectral data. The known – capsidol – showed significant *in vitro* bacteriostatic activity comparable to that of standard drug. Some of the isolated compounds were evaluated for antioxidant potential (Simona *et.al*.,2006).

Materials and Methods:-

Preparation of plant extract for phytochemical screening:-
(150 g) of powdered shade- dried fruits of *Capsicum annum* were macerated with n-hexane until exhaustion. This prepared extract(PE) was used for phytochemical screening according to the method described by Harborne(2001)

Extraction of oil from *Capsicum annum* seeds:-
Powdered seeds of *Capsicum annum* (200g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^oC for further manipulation.
Esterification of oil:-
A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol.A stock solution of methanolic sulphuric acid was prepared by mixing (1ml)of concentrated sulphuric acid with (99ml) methanol.

The oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid.The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes .The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate (1µl) was injected in the GC-MS vial.

GC-MS analysis:-

Capsicum annum seed oil was analyzed by gas chromatography – mass spectrometry.A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used.Helium (purity; 99.99 %) was used as carrier gas.Oven temperature program is given in Table 1, while other chromatographic conditions are depicted in Table 2.

Table 1:- Oven temperature program.

Rate	Temperature(^{o}C	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table 2: -Chromatographic conditions.

Column oven temperature	150.0^{o}C
Injection temperature	300.0^{o}C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec
Linear velocity	47.2cm/sec
Purge flow	3.0ml/min
Spilt ratio	- 1.0

Antimicrobial assay:-

Preparation of bacterial suspensions:-

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^{8}-10^{9}colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Preparation of fungal suspensions:-

Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity:-

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two

halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a sample. Separate Petri dishes were designed for standard antimicrobial chemotherapeutic agents. (ampicillin , gentamycin and clotrimazole).

The agar discs were removed, alternate cups were filled with (0.1) ml samples using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

Results and Discussion:-

Phytochemical screening:-

Phytochemical screening of *Capsicum annum* fruits gave positive reactions for: steroids,flavonoids,tannins,terpenes and glycosides(Table 3).

Table 3:- Phytochemical screening of *Capsicum annum* fruits.

Species	Flavonoids	Tannins	Steroids	Terpenes	Glycosides
Capsicum annum fruits	+ve	+ve	+ve	+ve	+ve

GC-MS analysis of *Capsicum annum* fixed oil:-

Constituents of *Capsicum annum* seed oil were identified and quantified by comparison with the MS library (NIST) .The fragmentation pattern resulting from GC-MS analysis was also studied. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

Constituents of oil:-

GC-MS analysis of the studied oil revealed the presence of 27 components(Table 4).The typical total ion chromatogram(TIC) of hexane extract is shown in Fig.1.

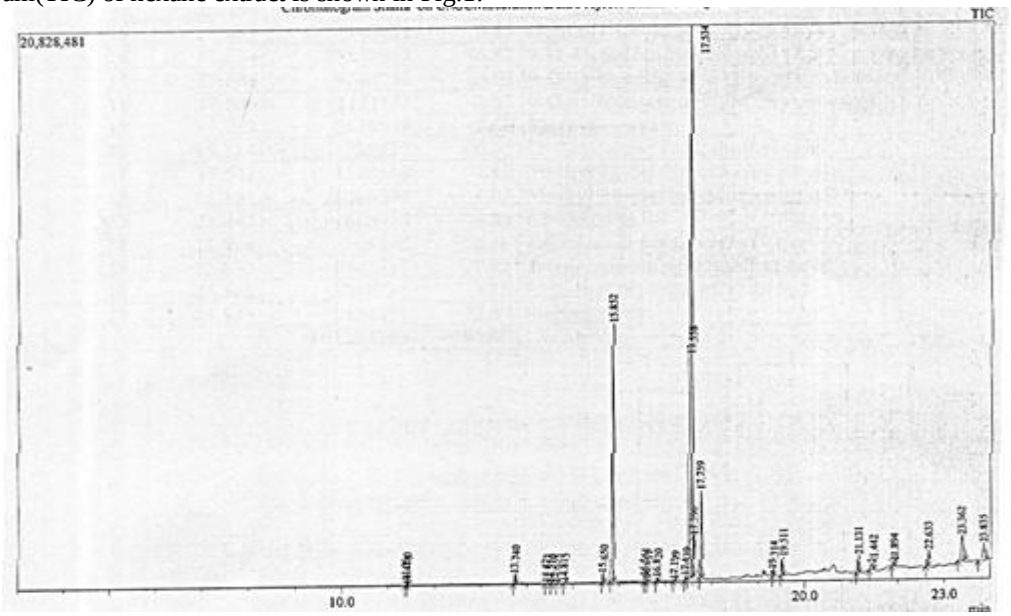

Fig.1:- Cromatograms of *Capsicum annum* seed oil.

Table 4:- Constituents of *Capsicum annum* fixed oil.

Peak	R.Time	Area	Area %	Name
1	11.393		0.22	Butylated hydroxytoluene
2	11.415		0.03	Dodecanoic acid,methyl ester
3	13.740		0.54	Methyl tetradecanoate
4	14.423		0.02	Hexadecenoic acid ,methyl ester
5	14.516		0.02	Tridecanoic acid ,12-methyl,methyl ester
6	14.550		0.01	5-octadecanoic acid,methyl ester
7	14.655		0.01	4-octadecenoic acid ,methy ester
8	14.815		0.02	Pentadecanoic acid,methyl ester
9	15.650		0.47	9-Hexacecenoic acid,methyl ester,Z
10	15.852		17.60	Hexadecanoic acid , methyl ester
11	16.551		0.25	Hexadecanoic acid,14-methyl,methyl ester
12	16.611		0.16	Methyl 5,13-docosadienoate
13	16.820		0.23	Heptadecanoicc acid, methyl ester
14	17.199		0.06	7-octadecenoic acid, methyl ester
15	17.439		0.33	Heptadecanoic acid,6-methyl, methyl ester
16	17.534		56.57	9,12-octadecadienoic acid, methyl ester
17	17.558		5.62	9-octadecenoic acid(z) , methyl ester
18	17.596		2.07	9-octadecenoic acid, methyl ester(E)
19	17.759		5.50	Methyl stearate
20	19.314		0.32	11-Eicosenoic acid, methyl ester
21	19.511		1.12	Methyl 18-methylnonadecanoate
22	21.131		1.05	Methyl 20-methylnonadecanoate
23	21.442		0.81	Cholesterol
24	21.894		0.26	Tricosanoic acid,metheryl es
25	22.633		1.02	Tetracosanoic acid, methyl ester
26	23.562		3.28	Ergost-8(14)-en-3-ol, (3-beta)-
27	23.835		2.37	Stigmasterol
			100.00	

The following compounds were detected in the chromatogram as major constituents:

9,12-Octadecadienoic acid methyl ester(56.57%)

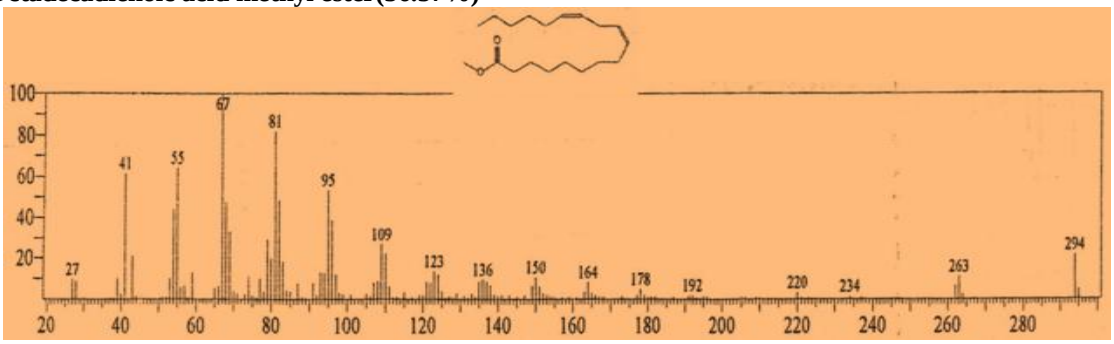

Fig. 2:- Mass spectrum of 9,12-octadecadienoic acid methyl ester

The peak at m/z 294, which appeared at R.T. 17.534 in total ion chromatogram(Fig.2) corresponds to $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 corresponds to loss of a methoxyl function.

Hexadecanoic acid , methyl ester(17.60%)

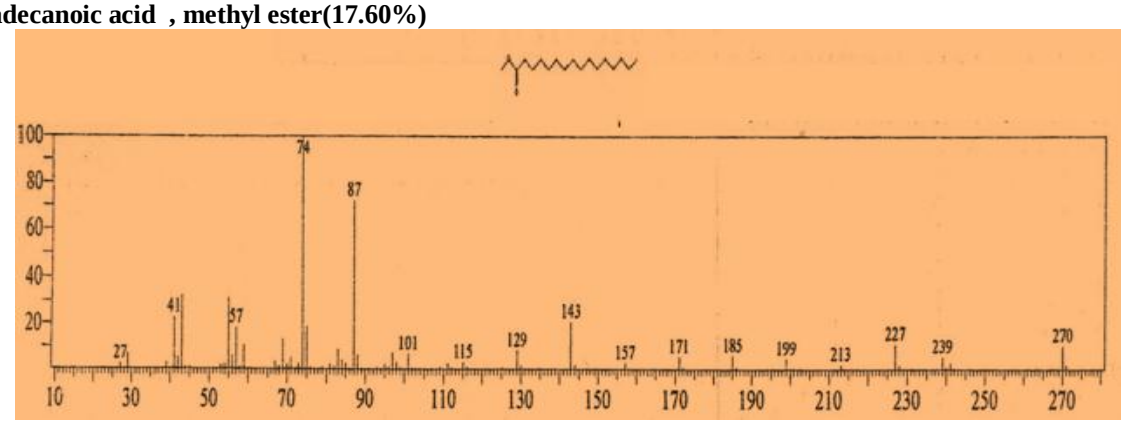

Fig. 3:- Mass spectrum of hexadecanoic methyl ester.

The EI mass spectrum of hexadecanoic acid methyl ester is displayed in Fig. 3. In total ion chromatogram, the peak at m/z 270 (R.T. 15.852) corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

9-Octadecenoic acid (Z) methyl ester(5.62%)

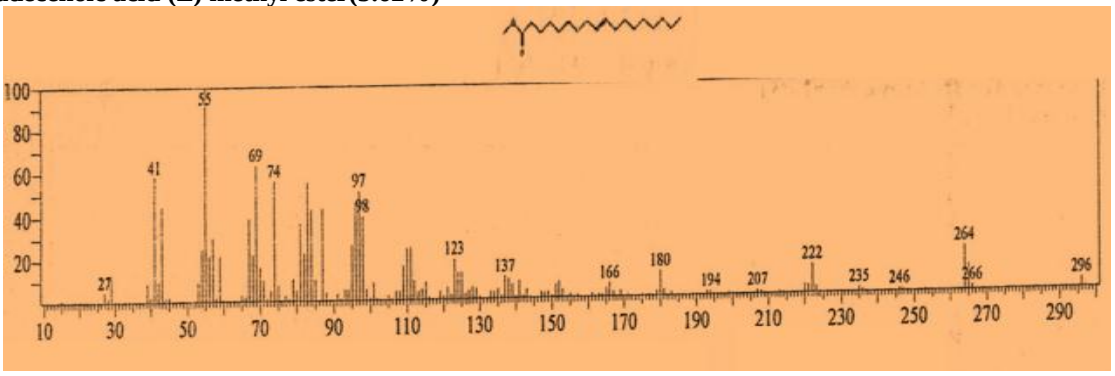

Fig. 4:- Mass spectrum of 9-octadecenoic acid methyl ester

The EI mass spectrum of 9-octadecanoic acid methyl ester is depicted in Fig. 4.The peak at m/z 296, which appeared at R.T. 17.558 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$ .The peak at m/z265 is due to loss of a methoxyl function.

Methyl stearate(5.50%)

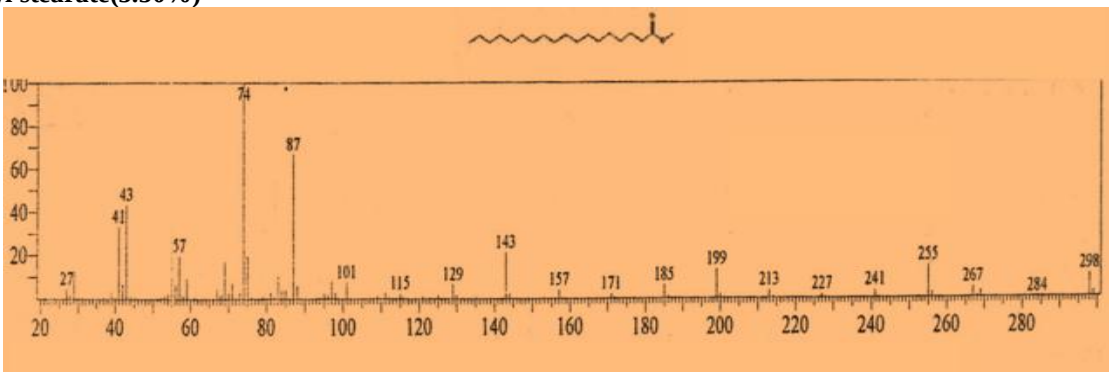

Fig. 5:- Mass spectrum of methyl stearate

Fig.5 shows the mass spectrum of methyl stearate .The peak at m/z 298 (R.T. 17.759) in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$.

Stigmasterol(2.37%)

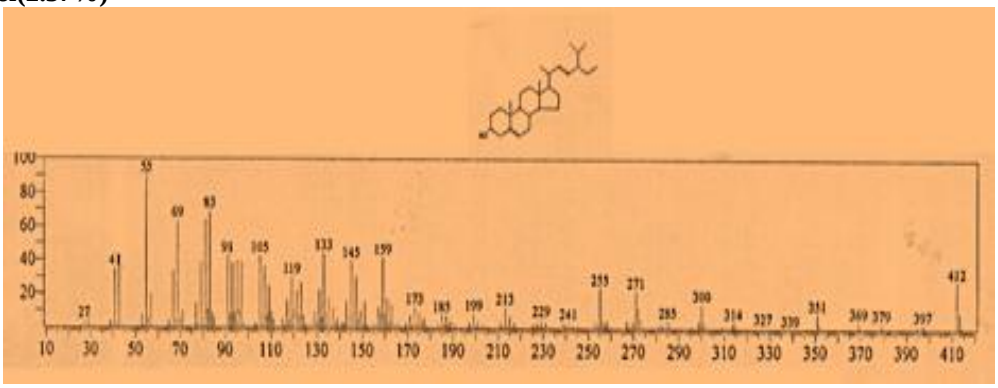

Fig 6:- Mass spectrum of stigmasterol

The peak at m/z 412 (Fig.6) which appeared at R.T.23.835 in total ion chromatogram, corresponds to $M^+[C_{29}H_{48}O]^+$.

Antimicrobial activity:-

Capsicum annum fixed oil was evaluated for antimicrobial activity against standard organisms. The average of the diameters of the growth inhibition zones are depicted in Table (5) .The results were interpreted in commonly used terms: (<9mm: inative; 9-12mm:partially active; 13-18mm: active; >18mm:very active) .Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 5:- Antibacterial activity of *Capsicum annum* oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Capsicum annum oil	100	-	15	24	15	9	13

Table 6:- Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 7:- Antifungal activity of standard chemotherapeutic agents against standard fungi.

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.: *Staphylococcus aureus*
- Ec.: *Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillus niger*
- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*

The oil showed significant activity against *Staphylococcus aureus*. It was also active against *Pseudomonas aeruginosa* and *Bacillus subtilis.* Though active against the fungal strain *Aspergillus niger,* it was patially active against the fungus *Candida albicans*. However, no activity was observed against *Escherichia coli*.

References:-

1. **Andrews,J**.(1984) " Peppers : The Domesticated Capsicum", University of Texas Press, Austin,USA.
2. **Alice,**M.,Federico,F.,Francisco,A.,Maria,I(2004) , *Agricultural and Food Chemistry Journal*,**52(12),**3861.
3. **Aneta**,W.,Dorota,J.,Monika,G.(2011) , *Acta Sci.Pol.Hortorum Cultus*,**10(1),**171.
4. **Francis**, J. K.(2013) "Capsicum annuum L. bird pepper , Issue of USDA Forest Service"
5. **Hayman**,M.,Kam,P.C.(2008),*Current Anaesthesia and Clinical Care* ,**19**,338.
6. **Harborne**, J. B(2001). "Phytochernical Methods", Chapman and Hall Ltd, London
7. **Keiko**,S.,Mayumi,M.,Keiko,I.,Kiniko,T.,Osamu,N.(2007), *Food Sci. Technol. Res*.,**13**(1),77
8. **McLeod**,M.J.,Eshbaaugh,W.H. and Gutterman,S.I.(1982) ,*Economy Botany*,**36**,361.
9. **Minguez** Mosquera, M. I.; Hornero Mendez, D.(1994), *Journal of Agricultural and Food Chemistry* **,42(7),** 1555.
10. **Oboh**,G.,Puntel,R.,Rocha,J.(2007), *Food Chemistry*,**102**(1),178.
11. **Rosa,**M.,Ana,J.,Paloma,R.,Felix,R.,Sierra,B.,Marina,L.,Manuel,G.,Francisca,S.,Luis,A.,Francisco. And Jose,M.(2013), *Int. J. Mol. Sci.*,**14**,9556.
12. **Simona** De Marino, Nicola,B.,Fulvio,G.,France,Z.,Gelsomina,F.,Rita,P. and Maria, L(2006), *Journal of Food Chemistry*,**54**(20),7508.
13. **Zhi-Yun** Z., Anmin L.and William G(2005). , *Flora of China*,**17**, 313.