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PRELIMINARY PHYTOCHEMICAL SCREENING, SPECTROSCOPIC STUDIES AND ANTIMICROBIAL SCREENING OF *CARTHAMUS TINCTORIUS* L.

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ABSTRACT: In present study the crude methanolic and aqueous extract of *Carthamus tinctorius* leaves were analyzed by different spectroscopic methods for exploration of medicinally important constituents and the studies also involves antimicrobial screening of *Carthamus tinctorius*. The spectroscopic analysis of *Carthamus leaves* by UV- Visible and FTIR were confirming the presence of many functional groups. The preliminary phytochemical studies of plant leaves revealed the presence of various useful phytoconstituents. The antimicrobial potential of plant leaves were investigated against six human pathogenic bacteria e.g., *Staphylococcus, E. coli, Pseudomonas, Streptococcus, Klebseilla and Bacillus cereus*. The aqueous extract of plant was found to be comparatively more active as an antimicrobial agent.

KEYWORDS: *Carthamus tinctorius*; FT-IR; UV-Vis; Phytochemicals; Leafy vegetable; Antimicrobial.

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1. INTRODUCTION

Plant serves as medicine since the time of antiquity, because of their lesser cost, no side effects, easy availability these herbal medicines becoming the need of both developed and developing countries. Plants is a source of huge varieties of secondary metabolites such as alkaloid, terpenoids and tannin which are reported to have antimicrobial properties. Since 1990s there is an emerging trend of using plant extract to treat health disorders because of awareness among the populations about the side

effects and microbial resistance of antibiotics. It was reported that around 440,000 cases of MDR TB are recorded annually which leads to the loss of about 150,000 deaths all around the world [1]. New phenotypes and new antimicrobial compounds are required to fight against these multiple drug resistance microbes [2]. In fact the theme of the World health day 2011 was Antimicrobial resistance: 'no action today no cure tomorrow'' [3]. A meeting of medical societies first ever in India was held for how to cope up with the problem of antimicrobial resistance in developing world in which the "Chennai declaration" came into practice and proceed to take efforts in this direction through a national and abroad policies[4]. The genetic capacity of transmit and acquire resistance against antibiotics is the root cause of multiple drug resistance in bacteria [5] bacteria transfer their antibiotic resistance to their progeny by vertical and horizontal transfer now there is urgency to develop a system against this problem [6]. The global rise of antibiotic resistant leads to occurrence of many health issues [7] According to the WHO report the causal bacteria of some life threatening diseases such as respiratory tract infections, diarrhea, meningitis, syphilis, gonorrhea and tuberculosis are more resistance [8]. The mechanisms by which bacteria becomes resistance are antibiotic inactivation [9] target modification [10] efflux mechanism of resistance [11], Plasmidic efflux [12]. Use of plants for human health care and for the preparation of drugs is very old trend and it considered to be a cheap one [13]. The antimicrobial property of plants are due to some components like aldehyde and phenolic groups of plants. The term Phytoalexins is used for broad number of diverse types of antimicrobial compounds present in plants or all kind of living organism for the survival against pathogens in the natural defense of plants [14]. For the purpose of living, growth and reproduction all organism alter and interconvert the large number of organic compounds. There is a need of make available themselves with the form of energy known as ATP and for manufacturing of their own tissues delivery of building block is required. They utilize a set of well regulated chemical reaction and incorporated enzymatic reaction for this function, which is together known as intermediary metabolism and pathway is commonly called as metabolic pathway. Living plants work like biochemical laboratory which construct primary and secondary metabolites from air, water, minerals and sunlight [15]. Primary metabolites are directly involved in normal growth, development and reproduction and secondary metabolites is derived from primary metabolites but are not directly takes part in the growth, development and reproduction but encompasses some ecological functions. The production of secondary metabolites is induced on the onset of particular type of environmental conditions or developmental stages [16]. Due to increasing demand of plants as an alternative medicinal source, the identification of phytoactives of plants responsible for their medicinal values becoming very significant [17-18]. For the screening of phytocomponents, spectroscopy methods are becoming more popular because they are cost effective, fast and simple to perform [19-20]. Fourier transform infrared spectroscopy is widely used to detect the structural information of photoactive of plants. UV-Vis spectroscopy use the light region from UV to visible

[21].The different colours of compounds are responsible for characteristic spectra of respective compounds, however the electronic transition plays a key role in this phenomenon [22]. India is called as a medicinal garden of the world in which Chhattisgarh state well known for the tribal diversity and traditional use of medicinal plants. Chhattisgarh state has 51 different types of leafy vegetables. One of the most wonderful members of world best garden of medicine is *Carthamus tinctorius*. *Carthamus tinctorius* is also known as bustard saffron; its seed oil is available in the market by the name Safflower [23]. In present study leaves of *Carthamus tinctorius* were used as a natural source antimicrobial agent and its antimicrobial effect were evaluated against some human pathogens such as *Esherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus cereus, Streptococcus pneumonia, Staphylococcus aureus.*

2. MATERIALS AND METHODS

2.1. Collection of plant material

The leaves of the *Carthamus tinctorius* were collected from the sabji market of Bilaspur, Chhattisgarh.

2.2. Sample extraction

The dry leaf powder of plant was allowed to successively extracted with pet ether, chloroform, ethyl acetate, acetone, methanol and water and allow to concentrated in a rotary evaporator at reduced pressure in the same order (non – polar to polar).

2.2.2 Phytochemical analysis

The qualitative analysis for investigating the presence of various phytochemicals were performed according to the method given by [24-25] with certain modifications.

1. Test for Alkaloids

a) Dragendorff's test- To the 1 ml of extract add few drops of Dragendorff's reagent slowly, If the reddish brown precipitate is observed then it indicates the presence of alkaloids in the sample.

b) Mayer's test- To the 1 ml of extract add little amount of Mayer's reagent, the appearance of cream coloured precipitate indicates the presence of alkaloids in the sample.

c) Wagners test: - Take 1 ml of extract and add few drops of Wagner's reagent, the appearance of reddish brown precipitate indicates the presence of alkaloids.

d) Hager's test: - To the 1 ml of extract add few drops of Hager's reagent, the formation of precipitate indicating the presence of alkaloids in the sample.

2. Test for Amino acids

a) Million's test – To the 1 ml of extract add 1 ml of millions reagent, formation of white coloured precipitate indicates the presence of amino acids in the sample.

b) Ninhydrine test – To the 1 ml of plant extract add few drops of Ninhydrine solution slowly then allow to boil, the presence of blue colour indicates the presence of amino acids in the plant extract.

3. **Test for Carbohydrates**

a) Molisch's test: To the 1 ml of extract add some drops of alcoholic α- naphthol, slowly add sulphuric acid through the side of the test tube, at the junction the formation of purple to violet colour rings appeared which indicates towards the presence of carbohydrates in the sample**.**

b) Benedict test: To the 1 ml of the extract add few amount of Benedict regent then allow to heat for sometimes, orange colour appears confirms the presence of carbohydrates.

c) Fehlings test: To the extract add 1 ml of Fehling's A and B and allow to heat in a water bath for 5-10 min. The appearance of reddish brown precipitate indicates the presence of carbohydrates.

4. **Test for Phenolic compounds (Tannins)**

a) Ferric chloride test: To the 1 ml of plant extract add half ml of ferric chloride solution, blue color indicates the presence of hydrolysable tannins and green colour indicates the presence of condensed tannins in the sample.

b) Gelatin test: To the 1 ml of plant extract add 1% solution of gelatin having 10 % sodium chloride. The formation of precipitate indicates the presence of phenolic compounds in the extract.

5. Test for Saponin

Froth formation test: Take 2 ml of extract and add 5 ml water then shake properly to form the froth if the froth is stable for 2 minutes then it indicates the presence of saponin in the extract.

6. Test for glycosides

a) Keller-killiani test: To the 1 mg of extract add 2 ml of chloroform then add 0.4 ml of glacial acetic acid with few drops of ferric chloride. Then add 0.5 ml of concentrated sulphuric acid carefully by the side of the test tube. The layer of acetic acid turns blue indicates the presence of glycosides in the extract.

b) Baljet's test: To the 2 ml of extract add 1 ml of picric acid solution, if orange colour appears indicates the presence of glycosides.

7. Flavonoids test

a) Shinoda test: To the 1 ml of extract add some magnesium turnings and then add concentrated hydrochloride drop by drop wait for few minutes, light pink, cream red or sometimes green or blue colour appears indicates the presence of flavonoids in the extract**.**

8. Lead acetate test – To the 1ml of extract add some drops of sodium hydroxide solution, intense yellow colour appears which get colorless after adding dilute acid drop wise which indicates the presence of flavonoids.

9. Test for terpenoids

a) Libermann –Burchard test: To the 1 ml of the extract add 5 ml of chloroform and add H_2SO_4 by the side of the test tube, dark green colour appers confirms the test for terpenoids.

b) Salkowaski's test: To the 1 ml of extract add few drops of H_2SO_4, wine red colour appears confirms the presence of terpenoids.

2.2.2. FT-IR

Fourier transform infrared spectrophotometer (FTIR) is used to analyze the presence of functional groups in the leaf extract. For this, a very small amount of sample was mixed in potassium dichromate. This mixture was properly mixed with morter to form a thin disc and placed in a sample cup for the reflectance. To obtain IR spectrum, Shimadzu affinity 2 spectrophotometer were used in the range of 4000 to 400 cm^{-1}. The data obtained were interpreted for the presence of functional groups [26-27].

2.2.3. Ultraviolet Visible Spectrophotometery

After shade drying, 2 gm powder of plant leaves extracted with the 50 ml of solvents with slow stirring for 72 hrs. After 72 hrs the solution was filtered with whatmann filter paper no 1, the collected filtrate subjected for UV –Vis analysis. For the analysis briefly, the leaf extract was centrifuged at 3000 rpm for 10 minutes. After filtration the sample dilutes 1:10 with the methanol then scanned with spectrophotometer instrument systronic-2203 from 200-1100 nm [28].

2.2.4. Antimicrobial screening

The aqueous and methanolic leaf extracts were screened to evaluate antibacterial efficiency of leaf against three gram positive and three gram negative bacteria which are human pathogenic in nature. The activity were performed by well diffusion method by following the method of [29] Bauer et al., 1966. The bacterial cultures were maintained in a nutrient agar media. For the maintenance the media were prepared by dissolving 28.0 gm of readymade media in 1000ml of distilled water. This suspension were allowed to heat for the proper mixing. Then media were autoclaved at 121ºC for 15 min at 15 lbs pressure. The media was allowed to cool and poured into the sterile test tubes in a laminar air flow. Then the test tubes were allowed to solidify. When the tubes were half solidify 4-5 colonies of bacteria from the respective pure culture were put into the test tubes of media then again the tubes were allowed to solidify. Broth solution incubated at 37± 1ºC for 2-8 hours in an incubator. To perform the antibacterial activity muller hinton agar (Himedia, India) were used. For media preparation, 38 gm of readymade media was allowed to suspend in a 1 litre of distilled water and the prepared suspension was allowed to put in a microwave oven for the proper mixing of media by melting. The media were autoclaved at 121ºC and pressure of 15 lbs for the 15 minutes. Then the autoclaved media were kept for cooling and then put all the materials into laminar air flow for the sterilization. The media were slowly poured into the sterile petriplates. Now the media containing petriplates were allow to solidify before use. Then take the inoculums and with the help of loop allowed to streaking on the entire surface of solidify petriplates of media. Then the plates was kept to solidify at room temperature for 15 min. In the above prepared plates 6mm agar well were prepared by using well punch. The 25µl of both the plant extracts equivalent to 500 mg per ml concentration of plant dry weight were put into the prepared wells of the plates. Ampicillin and amikacin were used as control antibiotic for the gram negative and gram positive bacteria respectively. Now the plates were incubated for 48 hours of time period at 37±1ºC in a incubator. After the incubation period

plates were observed for the inhibition of bacterial growth by the extracts. And the results were calculated by measuring the diameter of zone of inhibition of extract around the well by the help of zone measurement scale in millimeters.

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

The phytochemical analysis of *Carthamus tinctorius* leaf methanolic extract revealed the presence of many useful phytoconstituents such as alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, terpenoids, proteins, oil and carbohydrate. Alkaloids are reported to posses antihelmentic, antimicrobial and antidiarrheal activities, flavonoids having antimicrobial, antidiarrheal and antioxidative activity. Phenols and tannins are reported to have antioxidative activity along with antimicrobial, antidiarrheal and antihelmintic effects, glycosides having antidirreal effect, terpenoids also having antihelmentic, antimicrobial, and antidirreal activity and saponins having antidiarrheal, anticancer and antihelmentic activity.

Table1: Qualitative phytochemical analysis of *Carthamus tinctorius* leaf methanolic and aqueous extracts

Chemical test	Methanol	Aqueous
1 **Flavonoids**		
Alkaline reagent test	+	++
Lead acetate test	+	+++
Shinoda test	++	+++
2. **Phenolics**		
Ferric chloride test	+++	+++
Gletin Test	++	++
3.**Terpenoids**		
Libermann	++	++
Burchard test	++	+
Salkowaskis test	+++	++
Copper acetate test	++	+++
4**. Glycosides**		
Keller Killaini test	+	+
Beljets Test	+	+
6.**Alkaloids**		
Hagers test	+++	
Wagners test	++	++
Dragendoffr's test	++	+++

7. **Proteins** Ninhydrine test	++	++
8. **Carbohydrates** Molischs test Benedict test Fehlings test	 +++ ++ +++	 +++ ++ +++
9.**Test for Saponins**	++	+

+, +++ and ++ shows the low, high and very high intensity of presence of phytoactives in the extracts And – sign indicates the absence of phytoactives in the extract.

3.2 Spectroscopic analysis

The qualitative UV-VIS spectrum of methanolic extract was selected at wavelength of 400 to 700nm due to proper peaks. The fingerprint profile of methanolic extract showed peak at 663.2, 664, 420, 280, 278, 320, 400, 880, 665, 1100, 228.5, 665.2 nm with absorbance at 0.732, 0.027, 2.486, 0.26,4.00, 378, 360, 920, 0.028, 1043, 4.4034, 0.743, respectively. The profile of aqueous extract showed peak at 978.5, 672.5, 304, 342, 289, 976, 675, 401.6 nm with absorbance at 0.191, 0.25, 1.654, 0.422, 0.014, 0.191, 0.250, 1.987, respectively

Table 2: UV-VIS peak values of methanolic extracts of *Carthamus tinctorius* L.

S.N0	nm	Absorbance
1.	663	0.732
2.	664	0.027
3.	420	2.486
4.	280	0.26
5.	278	4.00
6.	320	378
7.	400	360
8.	880	920
9.	665	0.028
10.	1100	1043
11.	2285	4.4034
12.	665	0.743

Table 3: UV- VIS peak values of aqueous extracts of *Carthamus tinctorius* L.

S. No	nm	Absorbance
1.	978	0.191
2.	672	0.250
3.	304	1.654
4.	342	0.422
5.	289	0.014
6.	976	0.191
7.	675	0.250
8	401.6	1.987

3.3. FTIR Analysis

FT-IR analysis was performed to detect the presence of different functional groups in plant extract; its interpretation was based on peak values in the infrared region. This spectroscopic analysis helps in the identification of present chemical constituents and their structure elucidation. The aqueous extract exhibited characteristic absorption band 3498.87 (Alcohols, phenols), 2310.72 (C= Aldehydes), 2125.5 (-C=C- Stretch alkynes), 2125.5 (-C=C- Stretch alkynes), 1643.35(N-H bend 1º amines), 1554.63(C-C stretch in ring Aromatics), 1411.89,(C-C stretch in ring Aromatics), 790.81 (C- Cl stretch alkyl halides). Unfortunately, some peak values and their functional groups were not identified. The methanolic leaf extract showed characteristic bands at 3390.86 (2º N-H stretch 1º ,amine, esdiam 2974.23 (C-H stretch alka), 2893.22 (C-H stretch alkanes), 2538.32 (Unknown), 2137(-C=C-Stretch alkynes), 1921.10 (Unknown), 1651.(N-Hbends 1ºAmines), 1446(C-C stretch aromatics),1384(Unknown.) 891327.03(C-N stretch aromatic amines),1273.02(C-H wag Alkyl halides).

Table 4: FTIR peak values and functional groups of aqueous extract of *Carthamus tinctorius* L.

S.No	Peak areas	Mode of vibration	Functional group
1.	3498.87	C	Alcohols, phenols
2.	2310.72	C=	Aldehydes
3.	2125.5	-C=C-	Alkynes
4.	1643.35	N-H bend	Primery amines
5.	1554.63	C-C	Aromatics
6.	1411.89	C-C	Aromatics
7.	790.81	C-Cl	Alkyl halides

Table 5: FTIR peak values and functional groups of methanolic extract of *Carthamus tinctorius* L.

S.No	Peak areas	Mode of vibration	Functional group
1.	3990	N-H stretch	Primary and secondary amines, amides
2.	2974.23	C-H stretch	Alkanes
3.	2893.22	C-H stretch	Alkanes
4.	2538.32	Unknown	Unknown
5.	2137.13	-C=C- stretch	Alkynes
6.	1921.10	Unknown	Unknown
7.	1651.07	N-H stretch	Primary amines
8.	1446.61	C-C stretch	Aromatics
9.	1384.89	Unknown	Unknown
10.	1327.03	C-N stretch	Aromatic amines
11.	1273.02	C-H wag	Alkyl halides

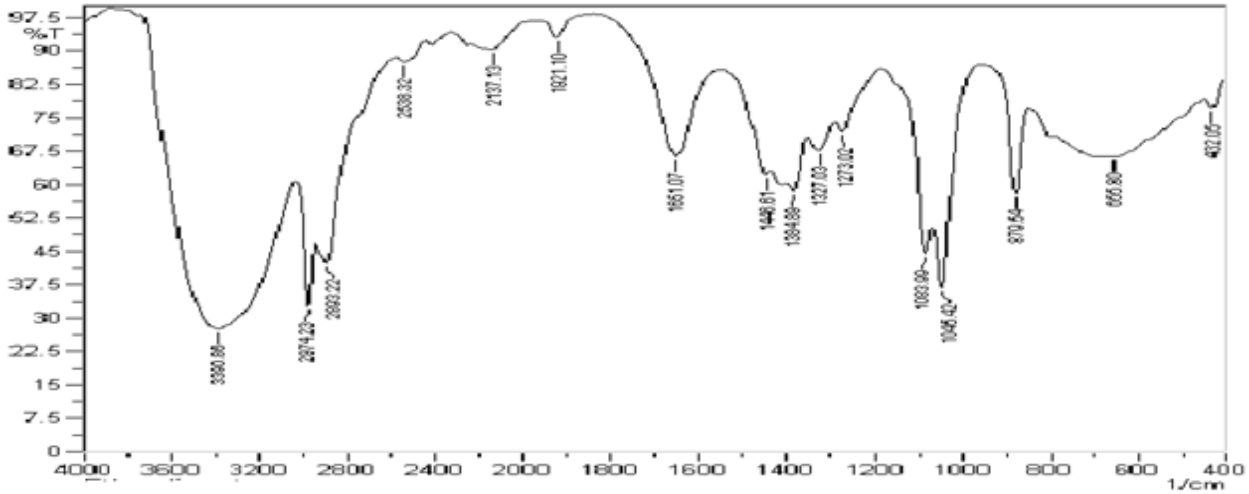

Fig 1: FTIR spectroscopic analysis of Methanol extract of *Carthamus tinctorius* leaves.

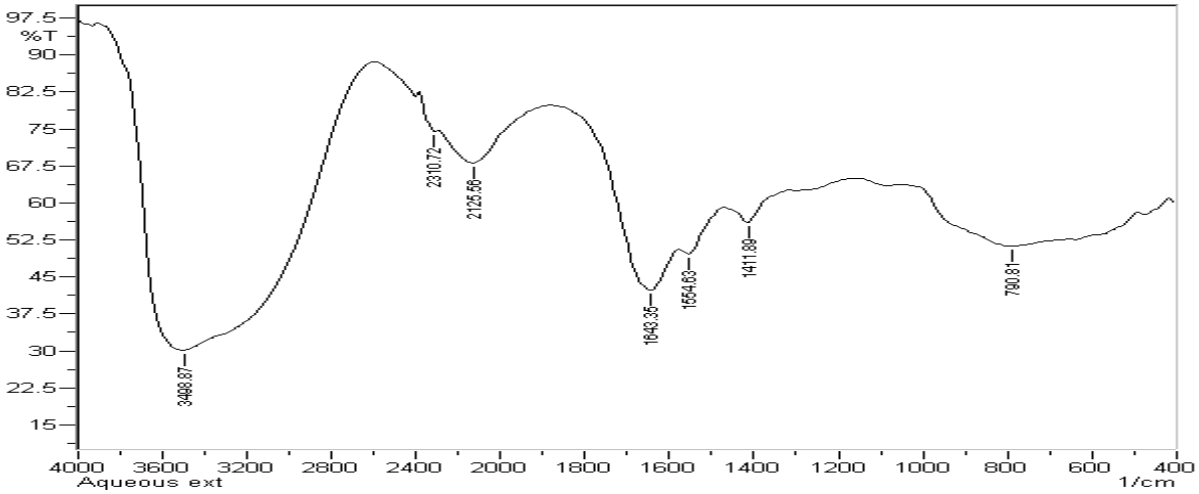

Fig 2: FTIR spectroscopic analysis of aqueous extract of *Carthamus tinctorius* leaves.

3.4. Antimicrobial activity of extracts

The antimicrobial activity were observed at stock solution of 500 µg/ml and at the three different dilutions (10^{-1},10^{-2} and 10^{-3}) of this stock. The antimicrobial activity of *Carthamus tinctorius* leaf extract six pathogenic bacterial species were selected namely *E.coli, Pseudomonas, Klebsiella, Bacillus, Staphylococcus and Streptococcus*. Against all the bacteria, gentamycin drug was used as a positive control. *Streptococcus* was found to resistant against both of plant extracts. Whereas, *E. Coli* (Zone size 19±1.15mm), *Pseudomonas* (19±1mm) and *Klebsiella* (23.33±1.527mm) were found to be sensitive in methanolic and aqueous extracts, respectively. *Bacillus* strain was found to be as intermediate (14±1mm).The activity was shown in table 6 and 7. There are various kinds of extrinsic and intrinsic factors which are responsible for the sensitivity of organism against the extract, like diffusion in the medium and permeability in the microbial cell [31]. Our findings suggest that aqueous extract having more potential as an antimicrobial compound than the methanolic extract and these finding supports the result of Mehrabian and Ramzi they reported in their studies that in comparison to other solvents aqueous extract of *Carthamus tinctorius* shows more bactericidal activity [32].

Table 6: Antimicrobial activity of methanolic leaf extract of *Carthamus tinctorius.*

Test strains	Gentamycin	Plant extract stock	PEd-1	PEd-2	PEd-3
E.coli	29	19.33±1.154	R	R	R
Pseudomonas auriginosa	16	R	R	R	R
Klebseilla pneumoneae	26	18.66±1.527	11±2	R	R
Bacillus	30	14±1.0	R	R	R
Staphylococcus	22	16.33±1.527	12±1	R	R
Streptococcus	18	R	R	R	R

R=Resistance, PEd= Plant extract dilution

Table 7: Antimicrobial activity of aqueous leaf extract of *Carthamus tinctorius*

Test strain	Gentamycin	Plant extract stock	PEd-1	PEd-2	PEd-3
E.coli	27	20±1.2	R	R	R
Pseudomonas	19	23.3±1.5	16.33±1.527	R	R
Klebseilla	28	20±1	14.33±1.527	R	R
Bacillus	26	19±1.1	10.33±1.527	R	R
Staphylococcus	24	R	R	R	R
Streptococcus	21	12.6±1.3	R	R	R

R=Resistance, PEd= Plant extract dilution

4. CONCLUSION

Present research suggesting the use of *Carthamus tinctorius* leaf, a leafy vegetable of Chhattisgarh as an antimicrobial agent to control the infectious diseases of humans. Both the extracts prove antimicrobially efficient. The isolation of active compounds from the leaves and their pharmacological trials in vivo and in vitro are required for the exploration of this leafy vegetable as medicaments. The qualitative phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, phenolics, saponins, terpenoids. The spectroscopic spectrum revealed the presence of antioxidative compounds caretenoids and beta cryptoxanthin and many important functional groups like alkenes, alkynes, aldehydes, nitro compounds, aromatics.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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