CHAPTER - 4

NEW OLEO-CHEMICALS FROM BY-PRODUCTS OF OIL PROCESSING
4.1 Introduction

4.1.1 Hydrazides

Hydrazides are the acylated derivatives of hydrazine and are usually encountered as the simple or mono-substituted (RCONHNH\textsubscript{2}), or as sym-di-substituted (RCONHNHOCR) compounds. The latter have been referred to as sec-hydrazide. Besides being useful themselves for a number of biological properties, hydrazides are important starting materials for a wide range of derivatives utilizable in pharmaceutical products and as surfactants. Their derivatives such as thiosemicarbazide possess useful pharmacological properties. Several triazole derivatives obtainable from hydrazides exhibit antibacterial activities in addition to fungicidal, insecticidal, and herbicidal activities. Oxadiazoles and their derivatives of hydrazides are well known chemotherapeutic agents. The condensation products of hydrazides and mono or poly saccharides possess good surfactant properties.

4.1.1.1 Physical Properties of Hydrazides

The mono-hydrazides of the normal saturated acids exhibit only a single melting point and no alternation between odd and even-numbered carbon homologs. The sym-di-hydrazides are unusual by virtue of the fact that an increase in chain length produces decrease in melting point. The limited data for the solubility of the hydrazides indicate that mono-hydrazides of the fatty acids are slightly more soluble in polar organic solvents and display fewer anomalies than the corresponding amides and those sym-di-hydrazides are considerably more insoluble in practically all solvents compared to their amide counterparts.
4.1.1.2 Preparation of hydrazides from oil

A variety of procedures have been developed to prepare hydrazides\(^7,8\). The most widely used method to prepare hydrazide is to treat the corresponding esters with hydrazine hydrate\(^9\).

\[
R
d\ \text{COOR'} + NH\text{H}_2 \leftrightarrow R\text{CONHNH}_2 + R'\text{OH}
\]

The reaction is reversible and product is sufficiently basic to react further with esters to yield sym-di-substituted product, particularly when the ester is in excess.

\[
R\text{CONHNH}_2 + R\text{COOR'} \leftrightarrow R\text{CONHNOCR} + R'\text{OH}
\]

The reactions involving unreactive esters generally require refluxing for few hours in a basic condition. It is a hazardous and energy intensive reaction and could evoke decomposition or degradation of the desired products.

A potential alternative to current technology is based on the use of biocatalysts. Enzymatic synthesis presents several advantages such as mild reaction conditions, no by products and significantly higher yield.

4.1.2 Schiff bases and their chemistry

Compounds containing an azomethine group (-CH=N-) are known as Schiff bases. They are usually formed by condensation of a primary amine with a carbonyl compound\(^10\) according to the following scheme.

\[
\begin{align*}
R\text{NH}_2 + R'\text{CH} & \rightarrow R\text{N}=\text{CHR'} + \text{H}_2\text{O} \\
\text{Primary amine} & \text{ Aldehyde} & \text{Schiff base}
\end{align*}
\]

where R may be an aliphatic or an aromatic group. Schiff bases of aliphatic aldehydes are relatively unstable and are readily
Polymerizable\textsuperscript{11-13} while those of aromatic aldehydes, having an effective conjugation system, are more stable\textsuperscript{14-17}. Condensations of amines with aldehydes and ketones have numerous applications which include preparative use, identification, detection and determination of aldehydes or ketones, purification of carbonyl or amino compounds, or protection of these groups during complex or sensitive reactions.

An amino group is found in simple amines and Schiff bases obtained from aromatic amines are known as anils. Schiff bases are generally bi- or tri-dentate ligands capable of forming very stable complexes with transition metals. In chemistry, Schiff bases find a versatile use\textsuperscript{18-20} some of them are the basic units in certain dyes, whereas, some are used as liquid crystals. In organic synthesis, Schiff base reactions are useful in making carbon-nitrogen bonds.

4.1.2.1 Biological importance of Schiff bases

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the increasing microbial resistance to antibiotics in use nowadays necessitates the search for new compounds with potential effects against pathogenic bacteria. The most spectacular advances in medicinal chemistry have been made when heterocyclic compounds played an important role in regulating biological activities.

Extensive investigations in the field of Schiff bases have been reported\textsuperscript{21,22}. Their preparation, chemical and physical properties have been described by various workers\textsuperscript{23,24}. Several workers have reported that Schiff bases formed from aromatic aldehydes or aromatic ketones and their derivatives are quite stable. Due to the great flexibility and diverse structural aspects of Schiff bases, a wide range of these compounds have been synthesized and their complexation behavior studied\textsuperscript{25,26}. Nitro and halo derivatives of
Schiff bases are reported to have antimicrobial and antitumor activities. Antimicrobial and antifungal activities of various Schiff bases have also been reported. Sahuet al. reported fungi toxicity of some Schiff bases. Gawadet al. synthesized some Schiff bases and observed high antimicrobial activities. Many Schiff bases are known to be medicinally important and are used to design medicinal compounds.

Schiff bases appear to be important intermediates in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most prevalent types of catalytic mechanisms in biochemical processes involves condensation of a primary amine in an enzyme, usually that of a lysine residue, with a carbonyl group of the substrate to form an imine or Schiff base.

Stereo chemical investigations carried out with the aid of molecular models showed that Schiff bases formed between methylglyoxal and the amino groups of the lysine side chains of proteins can bend back in such a way towards the N atoms of peptide groups that a charge transfer can occur between these groups and the oxygen atoms of the Schiff bases. In this respect, pyridoxal Schiff bases derived from amino acids have been prepared and studied. Schiff bases derived from pyridoxal and amino acids are considered very important ligands from the biological point of view. Transition metal complexes of such ligands are important enzyme models. The rapid development of these ligands resulted in an enhanced research activity in the field of coordination chemistry leading to very interesting conclusions.

Certain polymeric Schiff bases have been reported which possess antitumor activity. The Schiff bases have the highest degree of hydrolysis at pH 5 and the solubility in water is also highest at this pH. The antitumor activity of the bases towards ascetic tumours increases considerably with a slight increase in
water solubility. Another important role of Schiff base structure is in transamination\(^{40}\). Tranaminases are found in mitochondria and cytosol of eukaryotic cells. All the tranaminases appeal to have the same prosthetic group, i.e., pyridoxal phosphate, which is non-covalently linked to the enzyme protein.

The biosynthesis of porphyrin, for which glycine is a precursor, is another important pathway, which involves the intermediate formation of Schiff base between keto group of one molecule of 8-amino ievulinic acid and e-amino group of lysine residue of an enzyme.

### 4.1.3 Triazines

Triazines are three organic compounds, isomeric with each other, whose empirical formula is \(\text{C}_3\text{H}_3\text{N}_3\). Triazines are structurally a heterocyclic ring analogous to the six membered benzene ring but a three carbon compound replaced by nitrogens. The three isomer of triazines are distinguished from each other by the position of their nitrogen atoms, and are referred to as 1,2,4-triazines and 1,3,5-triazines.

![General structure of Triazines](image)

Substituted triazines represent an important class of nitrogen containing heterocycles. The 1,2,4-triazine core is a versatile synthetic platform to access a wide range of condensed heterocyclic ring systems via intramolecular Diels-Alder reactions with vast array of dienophiles\(^{41}\). Other aromatic nitrogen heterocycles are pyridines.
with 1 ring nitrogen atom, diazines with 2 nitrogen atoms in the ring and tetrazines with 4 ring nitrogen atoms, diazines with 2 nitrogen atoms in the ring and tetrazines with 4 ring nitrogen atoms. Triazines are weaker bases than pyridine\textsuperscript{41}.

The triazines ring system is a key component of commercial dyes, herbicides, insecticides, and more recently, pharmaceutical composition. Based on the concept that cationic charge, bulk, and lipophilicity are major factors determining antibacterial activity in anti microbial peptides, a set of compounds, identified to show potent antimicrobial activity together with low hemolytic activity have been designed and screened from several combinatorial libraries based on 1,3,5-triazine as a template\textsuperscript{42}.

The best known 1,3,5-triazine derivative is melamine with three amino substituents used in the manufacture of resins. Another triazines extensively used in resins is benzoguanamine. Triazine compounds are often used as the basis for various herbicides such as cyanuric chloride (2,4,6-trichloro-1,3,5-triazine). Chlorine substituted triazines are also used as reactive dyes. These compounds react through a chlorine group with hydroxyl group present in cellulose fibers in nucleophilic substitution, the other triazine position contain chromophores.

A series of 1,2,4-triazine derivatives known as BTPs have been considered in the liquid-liquid extraction community as possible extractants for use in the advanced nuclear reprocessing of used fuel. BTPs are molecules containing pyridine ring bonded to two 1,2,4-triazine-3-yl groups.

Although triazine are aromatic compounds the resonance energy is much lower than in benzene and electrophilic aromatic substitution is difficult but nucleophilic aromatic substitution more frequent.2,4,6-Trichloro-1,3,5-triazine is easily hydrolyzed to cyanuric acid by heating with water at elevated temperatures. 2,4,6-
Tris(phenoxy)-1,3,5-triazine reacts with aliphatic amines in aminolysis, and this reaction can be used to give dendrimers. Pyrolysis of melamine under expulsion of ammonia gives the tri-s-triazinemelem. Cyanuric chloride assists in the amidation of carboxylic acids.

The 1,2,4-triazines can react with electron rich dienophiles in an inverse electron demand Diels-Alder reactions. This form a bicyclic intermediate which normally then extrudes out a molecule of nitrogen gas to form an aromatic ring again. In this way the 1,2,4-triazines can be reacted with alkynes to form pyridine rings. An alternative to using an alkyne is to use norbornadiene which can be thought of as a masked alkyne.\textsuperscript{41}

4.1.3.1 Microwave-assisted solvent free synthesis of triazine derivatives

In the electromagnetic spectrum, the microwave radiation region is located between infrared radiation and radio waves. Microwaves have wavelengths of 1mm – 1m, corresponding to frequencies between 0.3-300GHz. Telecommunication and microwave radar equipments occupy many of the band frequencies in this region. In general, in order to avoid interference, the wavelength at which industrial and domestic microwave apparatus intended for heating operates is regulated to 12.2m, corresponding to a frequency of 2.45(+/- 0.05) GHz, but other frequency allocations do exist\textsuperscript{43}.

It has been known for a long time that microwaves can be used to heat materials. The heating effect utilized in these reactions is primarily due to dielectric polarization although conduction losses can also be important particularly at higher temperatures. Whilst the polarisability of a molecule is the sum of a number of contributions, only dipolar and interfacial polarization are important to the heating effect. When a molecule is irradiated, it rotates to align itself with the applied field. The frequency of rotation is similar to the frequency of
the applied radiation and consequently the molecules continually attempt to realign itself with the hanging field and energy is absorbed. The interfacial polarization, the Maxwell-Wagner effect, may also contribute to the heating effect when the conducting particles are in contact with a non-conducting medium. It is particularly convenient that qualitatively, the larger the dielectric constant, the greater the coupling with microwave\textsuperscript{44}.

The short reaction times and expanded reaction range that is offered by microwave assisted organic synthesis are suited to the increased demand in industries. In particular, there is a requirement in the pharmaceutical industry for a higher number of novel chemical entities to be produced, which require chemist to employ a number of resources to reduce production time\textsuperscript{45}. Other applications include sample preparation for analysis, waste treatment, polymer technology, drug release/targeting, ceramics and alkane decomposition and a range of decomposition processes including hydrolysis of proteins and peptides\textsuperscript{44}.

In general, most organic reactions have been heated using traditional heat transfer equipment such as oil baths, sand baths and heating jackets. These heating techniques are, however, rather slow and a temperature gradient can develop within the sample. In addition, local overheating can lead to product, substrate and reagent decomposition\textsuperscript{43}.

In contrast, in microwave dielectric heating the microwave energy is introduced into the reactor remotely and direct access by the energy source to the vessel is obtained. The microwave radiation passes through the walls and heats only the reactants and solvent, if present, and not the vessel itself. If the reactor is properly designed, the temperature increase will be uniform throughout the sample, which will lead to less by-products and/or decomposition of products. In pressurized systems, it is possible to rapidly increase
the temperature far above the conventional boiling point of the solvent\textsuperscript{43}.

The reaction is carried out as solid state reaction or non solid state reaction. Solid state reactions are of course very convenient from a practical view point, in general the solid support and reagents are efficiently mixed in an appropriate solvent which is then evaporated. The adsorbed reagents are then placed in a vessel and subjected to irradiation after which the organic products are simply extracted from the support. The absence of solvent coupled with the high yields and short reaction time often associated with these reaction make these procedures very attractive for synthesis\textsuperscript{44}.

1,2,4-triazines are a representative class of heterocyclic compounds with a wide variety of interesting properties which are used in medicine and agriculture. It has been associated with diverse pharmacological activities such as hypertension and inhibition of platelets, antileukemic, anti-inflammatory and potent neuroprotective agents. The 1,2,4-triazine moiety is a structural element in antimalarial, anticancer, antifungal, anticonvulsant, antibacterial and antiviral compounds. Certain compounds containing a 1,2,4-triazine nucleus have been reported to possess pesticidal, neuropharmacological, analgesic and antidepressant properties. Some 1,2,4-triazine derivative are used for the determination of metal ions and as dyes. N-methyl derivatives of 1,2,4-triazine are the naturally occurring antibiotics fervenulin (planomycin), toxoflavin (xanthothricin) and reumycin\textsuperscript{46}.

The design and synthesis of effective and potent antimicrobials is an area of immense significance for medicinal chemists. In this context, s-triazine derivatives have received considerable attention due to its potent biological activity such as anti-protozoals\textsuperscript{47}, estrogen receptor modulators\textsuperscript{48}, antimalarials\textsuperscript{49-50}, cyclin dependent kinase inhibitors\textsuperscript{51}, antivirals\textsuperscript{52}. It has been
reported that s-triazine derivatives possess potent antimicrobial activity\textsuperscript{53-57}.

Triazine derivatives, the compound of our interest can be synthesized by variety of methods. 1,2,4-triazines are prepared from condensation of 1,2-dicarbonyl compounds with amidrazones. A classical triazines synthesis is also the Bamberger triazine synthesis. Symmetrical 1,3,5-triazines are prepared by trimerization of cyanogen chloride or cynamide. Benzoguanamine (with one phenyl and two amino substituents) is synthesized from benzonitrile and dicyandiamide in dimethoxyethane with potassium hydroxide.

The traditional thermal conditions involve heating a 1,2-diketone and an acyl hydrazide, a 1:1 ratio, with excess ammonium acetate in refluxing acetic acid for 6-24 hours. Recently, ‘dry media’ microwave assisted protocols have emerged where in an inorganic support, Such as silica gel, is employed as the energy transfer medium in lieu of solvent\textsuperscript{53}. This approach has been applied to the preparation of 1,2,4-triazine to successfully deliver products in good yield using a conventional microwave oven. With the salient features of microwave assisted organic synthesis in mind, the current works aims at synthesizing triazines derivatives by microwave irradiation of hydrazide derivative of AO and ORSBE.
General scheme for Triazine synthesis.

R1 = Fatty alkyl chain
R2 = R3 = CH₃, Ph.
4.2 Present Work

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology as control tool for infectious diseases, however, the increasing microbial resistance to conventional antibiotics in use necessitates the search for new compounds with improved effects against pathogenic bacteria. The most spectacular advances in medicinal chemistry have been reached when heterocyclic compounds played an important role in regulating biological activities. Several workers have reported that Schiff bases formed from aromatic aldehydes or aromatic ketones and their derivatives are quite stable. Hydrazides, their various Schiff bases and triazine derivatives possess useful pharmacological properties along with antibacterial and antifungal activities.

Therefore the present work is aimed at exploring the possibility of preparing long chain fatty acid hydrazides, their various Schiff bases and triazine derivatives from acid oil and oil recovered from spent bleaching earth (ORSBE), a by-products of oil processing with a view to utilize it for value added applications to exploit its potential availability.
4.3 Literature Survey

- L. Kyme, G. S. Fisher and W. G. Bickford\textsuperscript{58} prepared hydrazides of 17 fatty acid from their esters by refluxing the esters for two hours with one mol of 43\% aqueous hydrazine hydrate per gm of ester together with sufficient ethanol to produce homogeneous solution at reflux temperature. They indicated that elaidic acid formed a hydrazide in good yield but oleic acid underwent reduction and yielded mainly the hydrazide of stearic acid. The hydrolysis of fatty hydrazide to produce corresponding fatty acid was also reported.

- G. M. Kinhikar, B. Y. Rao and C. V. N. Rao\textsuperscript{59} prepared oleic hydrazide by hydrazinolysis of methyl oleate in nitrogen atmosphere.

- R. C. Badami, S. B. Hendi and K. B. Patil\textsuperscript{60} reported preparation of long chain fatty hydrazides by reaction of fatty acid esters with hydrazine hydrate. The hydrazides were converted to different derivatives such as oxadiazoles, thiosemicarbazides and triazoles.

- H. A. Bhakere, R. R. Khotpal and A. S. Kulkarni\textsuperscript{61} carried out Studies on synthesis of oxadiazoles, thiosemicarbazides and triazoles from some fatty acid hydrazides. Hydrazides of lauric, myristic, palmitic, stearic, oleic, linoleic and chaulmoogric acids were cyclized to 2-alkyl-5-mercapto-1,3,4-oxadiazoles using carbon disulfide and potassium hydroxide. The hydrazides were also converted into thiosemicarbazides by reacting them with potassium thiocyanate in the presence of hydrochloric acid. The thiosemicarbazides on heating with potassium hydroxide solution were cyclized to 3-alkyl-5-mercapto-1,2,4-triazoles. Similarly the thiosemicarbazides were also cyclized to 2-alkyl-5-amino-1,3,4-oxadiazoles using iodide in potassium iodide solution.
Some new 2-alkyl-5-mercapto-1;3;4-oxadiazoles, 3-alkyl-5-mercapto-1,2,3-4H triazoles synthesized from hydrazides of neem oil and rice bran oil by S.D. Toliwal, K. Jadav and K. Patel\textsuperscript{62}. These newly synthesized compounds were characterized on the basis of elemental analysis and evaluated for biological properties. Certain derivatives exhibited fairly high antibacterial and antifungal activities when compared with streptomycin and imidil used as standard antibacterial and antifungal agent respectively.

S. D. Toliwal, K. Jadav, K. Patel and A. Banu\textsuperscript{63} synthesized 5-alkyl-[1,3,4]-oxadiazol-2-yl-phenyl amine, phenyl thiosemicarbazide, 5-alkyl-4 phenyl-4H-[1,2,4] triazole-3-thiol and 2-alkyl-5-phenylsulfanyl-[1,3,4]-oxadiazole from phenyl hydrazides of karanja oil. These newly synthesized compounds were characterized on the basis of elemental analysis. Some of the synthesized compounds exhibited fairly high antibacterial and antifungal activity.

S. D. Toliwal, K. Jadav, A. Gupte and A. Banu\textsuperscript{64} synthesized some new (5-alkyl-[1,3,4]-oxadiazol-2-yl)-phenyl amine, phenyl thiosemicarbazide, 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol and 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole from phenyl hydrazides of rice bran oil. These newly synthesized compounds were characterized on the basis of elemental analysis. Some of the synthesized compounds when tested exhibited fairly high antibacterial and antifungal activity when compared with standards.

S. D. Toliwal, K. Jadav and K. Patel\textsuperscript{65} made some new 2-alkyl-5-mercapto-1,3,4-oxadiazoles and 3-alkyl-5-mercapto-1,2,3-4H triazoles from hydrazides of acid oil and oil recovered from spent bleaching earth. These newly synthesized compounds were characterized on the basis of their elemental analysis and evaluated for biological properties. Certain derivatives exhibited fairly high antibacterial and antifungal activities when
compared with streptomycin and imidil used as standard antibacterial and antifungal agents respectively.

- S. D. Toliwal, K. Jadav, T. Pavagadhi, A. Gupte and A. Banu synthesized some schiff bases of fatty acid hydrazides made from nontraditional oils- Neem, Rice Bran and Karanja. These newly synthesized schiff bases were characterized on the basis of elemental analysis and evaluated for biological performance. Certain schiff bases fairly exhibited high antibacterial and antifungal activities when compared with streptomycin and imidil used as standard antibacterial and antifungal agents respectively.

- A. Rauf, S. Sharma and S. Gangal investigated rapid and efficient solvent-free synthesis of 3,5,6-trisubstituted-1,2,4-triazines from fatty acid hydrazides under microwave irradiation. The one-pot synthesis on solid inorganic support provided the products in good yields. The newly synthesized compounds were screened for antimicrobial activity. The structural features of the synthesized 1,2,4-triazines were characterized by IR, 1H NMR, 13C NMR, mass and elemental analysis.

- J. P. Raval, A. R. Rai, N. H. Patel, H. V. Patel and P. S. Patel synthesized a variety of N’-(4-(arylamino)-6-(pyrazin-2-ylamino)-1,3,5-triazin-2-yl)isonicotinohydrazide, by using 2-aminopyridine, isonicotic acid hydrazide and cyanuric chloride. The structures of these compounds were confirmed by IR, NMR (1H & 13C) spectral analysis. The newly synthesized compounds were also evaluated for antimicrobial activity against variety of bacterial strains and some of these compounds had shown significant antibacterial and antifungal activities.

- S. D. Toliwal, K. Jadav, B. Bhatt, N. Verma and N. Jha made enzymatic hydrazides from nontraditional oils by a one
step lipase catalyzed reaction, carried out by treating the oils with hydrazine mono hydrate at neutral pH using a lipozyme as the catalyst. The hydrazides were also made by chemical route from nontraditional oils by treating their methyl esters with hydrazine monohydrate. Rapid and efficient solvent free synthesis of triazines from chemically synthesized fatty hydrazides made from nontraditional oils under microwave irradiation was carried out using silica gel as an inorganic solid support. The structural features of the synthesized hydrazides and triazines were characterized by FT-IR and elemental analysis. The newly synthesized triazines and enzymatically made hydrazides exhibited fairly good antimicrobial activity.

- S. D. Toliwal, K. Jadav, A. Gupte and A. Banu investigated that hydrazones possessing an azomethine -NHN=CH- proton constitute an important class of compounds for new drug development. Therefore, many researchers synthesized these compounds as target structures and evaluated their biological activities. These observations were aimed for the development of new hydrazones that possess varied biological activities. In line with these observations, some new hydrazones synthesized from hydrazides of neem, karanja and rice bran oils with a view to impart value addition to these non-traditional oils. These newly synthesized compounds were characterized on the basis of elemental analysis and evaluated for biological properties. Certain hydrazones exhibited reasonable antibacterial and antifungal activities when compared with standards.
4.4 Materials and Methods
Oil recovered from spent bleaching earth (ORSBE) and Acid oil were procured from Ashwin Vanaspti Ltd, Samlaya, Gujarat, India. Concentrated sulfuric acids, sodium carbonate, needed for methyl ester preparation, were of laboratory grade. Hydrazine hydrate (assay 98%), KSCN and various aldehydes used were of laboratory grade for thiosemicarbazide and Schiff base preparation. Diketone, silicagel, ammonium acetate and ethylene triamine for preparation of triaziens were laboratory grade reagents. All other chemicals and materials used were laboratory grade reagents.

4.5 (A) Experimental

4.5(A).1 Preparation of Schiff bases

1. Preparation of methyl ester from oil

Methyl ester from oils were prepared by acid catalyzed esterification method in which 100 gm oil was taken in 500 ml round bottom flask and 300 ml methanol and 1ml concentrated sulfuric acid were added. The contents were refluxed for 4 hrs. on water bath. At the end of reaction, the excess methanol was distilled off and 50 ml distilled water was added. The contents were then transferred to separating funnel and lower aqueous layer was withdrawn. The upper organic layer was washed 2-3 times with 1% sodium carbonate solution to remove un-esterified fatty acid.

2. Preparation of fatty acid hydrazides

To a solution of a fatty acid esters (0.1M) in ethanol (150 ml) hydrazine hydrate (95%, 0.2M) was added. The reaction mixture was refluxed for 3-4 hrs. It was cooled, and the solid separated was collected, washed and recrystallized from ethanol.
3. Preparation of thiosemicarbazides

To a solution of a fatty acid hydrazide (0.02 M) in methanol (50 ml) a solution of potassium thiocynate (0.03 M) and hydrochloric acid 3 ml was added with constant stirring. The mixture was immediately evaporated to dryness on a steam bath and heated for an additional hour with another 50 ml ethanol. The resulting solid was treated with water, and with little ethanol and recrystallized from ethanol.

4. Preparation Schiff bases from thiosemicarbazides

Fatty thiosemicarbazides (0.02M) were added to a solution of aldehyde (0.02M) in absolute alcohol and was refluxed for 7 hrs. on a steam bath. The mixture was cooled, the solid separated was filtered and crystallized from alcohol.
The preparation of hydrazides and their Schiff bases are schematically shown as under

\[
\text{RCOOCH}_3 + \text{NH}_2\text{NH}_2 \rightarrow \text{RCONHNH}_2 \rightarrow \text{RCONHNHCSNH}_2
\]

**(Scheme 1)** – Preparation of fatty hydrazides and their Schiff bases.

**A-** Fatty acid hydrazides,

**B-** Thiosemicarbazide,
**C-** 2-alkyl-N-benzylidenehydrazinecarbothioamide, 

**D-** 2-alkyl-N-(3-methoxy-4-hydroxy benzylidene)hydrazinecarbothioamide, 

**E-** 2-alkyl-N-(furan-2-ylmethylene)hydrazinecarbothioamide, 

**F-** 2-alkyl-N-(4-hydroxybenzylidene)hydrazinecarbothioamide, 

### 4.5(A).2 Analysis and characterization of derivatives

#### 1. Determination of melting point

The sample was melted in a small weighing tube till it become liquid. A capillary sealed at one end was inserted in the melted sample till the liquid entered the capillary to 1” height. The capillary was then cooled in ice for 5 min to get the sample well solidified. The capillary was tied to 0-360°C thermometer and thermometer was immersed in melting point apparatus containing liquid paraffin. The melting point apparatus was heated slowly. The temperature at which the sample melted was noted. The melting points of derivatives are shown in Table 1.

#### 2. Determination of nitrogen content by Kjeldahl’s Method

About 1 to 2 gm of sample was weighed accurately in 250 ml Kjeldahl’s flask. 10 gm of potassium sulfate and 1 gm of copper sulfate and 35 ml concentrated sulfuric acid were added. The contents were digested for 45 min or till the liquid became clear and of very faint straw colour. The flask was then allowed to cool to room temperature and about 100 ml distilled water was added to dilute the contents. 20 ml of this diluted solution was taken for distillation and added to distillation apparatus. 10 ml, 0.5N standard sulfuric acid solution was taken in
conical flask, few drops of methyl red indicator were added and the flask was placed under condenser tip with tip of the condenser dipping well in to the acid solution. 10 ml of 50% NaOH was then run into the distillation apparatus and distillation was started. The liberated ammonia was absorbed in the acid solution. The distillation was carried out for 25 – 30 min or till volume of content in conical flask increased to about 30 ml. The acid solution was titrated against 0.5N standard sodium hydroxide solution. A blank determination was also conducted. Nitrogen content (Table 1) was calculated by the formula.

Nitrogen content (%) = 1.4 x (B – S) x N/W

Where,

N = Normality of sodium hydroxide solution used.

W = Weight of sample in gm

S = Volume of sodium hydroxide solution required for blank

B = Volume of sodium hydroxide solution required for sample

3. Infrared Spectra of hydrazides and their Derivatives

IR spectral measurement of organic derivatives is considered to be one of the most useful methods of characterization. In principle, it provides qualitative and quantitative information about the structural details of the compound under examination. The empirical information of IR spectra is based on the concept of nearly independently vibrating atomic groups in the macromolecule. IR spectra are measured either in the solid state in the form of KBr pallet or in the form of film or by preparing solution of sample after dissolving it in to suitable solvent. One of the most popular applications of IR spectral
study is detection of functional groups in the compound. In this study Infrared spectra were recorded on FT-IR spectrum GX (Perkin Elmer) infrared spectrometer in KBr. (Fig. 1-12)

4.5(A).3 Biological activities of Schiff Bases

1. Determination of anti-bacterial activity

Nutrient agar solution used was made with following composition:

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Peptone</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>Beef extract</td>
<td>0.3 gm</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.3 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>Distilled</td>
<td>100 ml</td>
</tr>
<tr>
<td>water</td>
<td></td>
</tr>
<tr>
<td>Agar agar</td>
<td>3 gm</td>
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<tr>
<td>bacto powder</td>
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The pH of the solution was adjusted between 7 – 7.2. The solution was sterilized in autoclave at 120 °C and 15-lb/in² pressure for 15 min. The sterilized solution was used for preparing nutrient agar plate. Soft agar tubes were made by adding sterile agar powder 1 gm in 100 ml distilled water in test tube. 0.1 ml of bacterial culture (0.5 optical densities) was inoculated into sterile melted soft tube and mixed well and overlaid onto nutrient agar plate. The contents were allowed to solidify. A cup borer was taken and sterilized by dipping in
alcohol and subjecting it to flame. The nutrient agar plate was divided in 4 sectors and a cup was bored in the centre of each sector. Each cup was filled with 0.1 ml solution containing 200 μg of the compound to be tested. The loaded plates were kept in refrigerator for 30 min for pre-diffusion and incubated at 37 °C for 24 hours. Next day the diameter of zone inhibition was measured in mm and compared with standard antibiotic.

**Selected Microorganisms:**

<table>
<thead>
<tr>
<th>Nature</th>
<th>Organism</th>
<th>Causal agent of…</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Positive</td>
<td><em>Bacillus subtilis</em></td>
<td>Food poisoning</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>Bacillus cereus</em></td>
<td>Food poisoning, vomiting, diarrhea.</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>Wound infection</td>
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<td></td>
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<td>Pneumonia.</td>
</tr>
<tr>
<td></td>
<td><em>Micrococcus luteus</em></td>
<td>Septic shock, septic arthritis.</td>
</tr>
<tr>
<td>Gram Negative</td>
<td><em>Escherichia coli</em></td>
<td>Bloody diarrhea, kidney disease.</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>Salmonella typhi</em></td>
<td>Typhoid, enteric fever.</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Septicemia, pneumonia, dermatitis.</td>
</tr>
<tr>
<td></td>
<td><em>Serratia Marcescens</em></td>
<td>Bacteremia, Urinary and respiratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infection</td>
</tr>
</tbody>
</table>
2. Determination of anti-fungal activity

Potato Dextrose agar solution used was made with following Composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato infusion</td>
<td>200 gm/ lit</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20 gm / lit</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>Agar Agar</td>
<td>50 gm / lit</td>
</tr>
</tbody>
</table>

The pH of the solution was adjusted between 5.0 – 5.5. The solution was sterilized in autoclave at 120 °C and 15-lb/in² pressure for 15 min. The sterilized solution was used for preparing Potato Dextrose agar plate. Soft agar tubes were made by adding agar powder 1.0 gm in 100 ml distilled water, melted, distributed 10 ml in test tubes and sterilized. 0.1 ml of fungal culture was inoculated into sterile melted soft agar tube and mixed well before being overlaid onto Potato Dextrose plate. The contents were allowed to solidify. A cup borer was taken and sterilized by dipping in alcohol and subjecting it to flame. The Potato Dextrose plate was divided in 4 sectors and a cup was bored in the centre of each sector. Each cup was filled with 0.1 ml solution containing 200 ppm of the compound to be tested. The loaded plates were kept in refrigerator for 30 min for pre-diffusion and incubated at 37 °C for 24 hours. Next day the diameter of zone inhibition was measured in mm and compared with standard antibiotic.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Causal agent of...</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>Thrush, vulvovagininitis, Cutaneous Candidiasis, Pulmonary candidiasis (69)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Allergic bronchopulmonary aspergillosis, Lung disease coughing blood, dry cough, wet cough, coughing spasms, persistent cough, chronic cough, severe cough, or other types. Fungal ear infection, ear pain, impaired hearing, ear inflammation tinnitus impaired lung function (70)</td>
</tr>
</tbody>
</table>
4.5(A).4 Results

Table 1.

Characterization of hydrazides, thiosemicarbazides and Schiff bases

<table>
<thead>
<tr>
<th>Sample</th>
<th>M. Wt. (gm/mole)</th>
<th>Melting Point (°C)</th>
<th>% Yield</th>
<th>Nitrogen Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td></td>
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<td>Found</td>
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<tr>
<td>OR1</td>
<td>270</td>
<td>86</td>
<td>65.8</td>
<td>10.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.42</td>
</tr>
<tr>
<td>AO1</td>
<td>242</td>
<td>87</td>
<td>80.3</td>
<td>10.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.36</td>
</tr>
<tr>
<td>OR2</td>
<td>329</td>
<td>92</td>
<td>60.63</td>
<td>12.76</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>8.69</td>
</tr>
<tr>
<td>AO2</td>
<td>301</td>
<td>94</td>
<td>78.00</td>
<td>12.76</td>
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<td>9.5</td>
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<tr>
<td>OB1</td>
<td>417</td>
<td>86</td>
<td>62.13</td>
<td>10.07</td>
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<td></td>
<td></td>
<td>8.52</td>
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<tr>
<td>OB2</td>
<td>463</td>
<td>83</td>
<td>65.17</td>
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<td></td>
<td>7.18</td>
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<tr>
<td>AB1</td>
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<td></td>
<td>10.15</td>
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<tr>
<td>AB2</td>
<td>435</td>
<td>111</td>
<td>55.08</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.45</td>
</tr>
<tr>
<td>AB3</td>
<td>405</td>
<td>96</td>
<td>62.08</td>
<td>9.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.91</td>
</tr>
<tr>
<td>AB4</td>
<td>389</td>
<td>92</td>
<td>80.2</td>
<td>10.31</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>9.86</td>
</tr>
<tr>
<td>OB3</td>
<td>433</td>
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<td>63.95</td>
<td>9.69</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>8.02</td>
</tr>
<tr>
<td>OB4</td>
<td>407</td>
<td>77</td>
<td>75.74</td>
<td>10.31</td>
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<td>9.00</td>
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</tbody>
</table>
### Table 2.

**Antibacterial activity of Schiff bases**

<table>
<thead>
<tr>
<th>Nature</th>
<th>Test organism</th>
<th>+ve control</th>
<th>-ve Control</th>
<th>OB₁</th>
<th>OB₂</th>
<th>AB₁</th>
<th>AB₂</th>
<th>AB₃</th>
<th>AB₄</th>
<th>OB₃</th>
<th>OB₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Positive</td>
<td><em>Bacillus subtilis</em></td>
<td>++</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus cereus</em></td>
<td>+++</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>+++</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td></td>
<td><em>Micrococcus luteus</em></td>
<td>++</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gram Negative</td>
<td><em>Escherichia coli</em></td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella typhi</em></td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Serratia Marcescens</em></td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Diameter of zone of inhibition <10 mm (+), 10-15 mm (++), 15-20 mm (+++), 20-25 mm (++++).

Concentration of tested compounds 200ppm, Streptomycin (200 ppm) used as +ve control, DMSO (Dimethyl Sulphoxide) used as –ve control.
Table 3.

Antifungal activity of Schiff bases

<table>
<thead>
<tr>
<th>Test organism</th>
<th>+ve control</th>
<th>-ve control</th>
<th>OB1</th>
<th>OB2</th>
<th>AB1</th>
<th>AB2</th>
<th>AB3</th>
<th>AB4</th>
<th>OB3</th>
<th>OB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Diameter of zone of inhibition <10 mm (+), 10-15 mm (++) , 15-20 mm (+++), 20-25 mm (++++) .

Concentration of test compounds 200ppm, Streptomycin (200 ppm) used as +ve control, DMSO (Dimethyl-Sulphoxide) used as –ve control.
## Derivative Code

<table>
<thead>
<tr>
<th>Code</th>
<th>Name of Derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR₁</td>
<td>Chemically synthesized fatty acid hydrazide of ORSBE.</td>
</tr>
<tr>
<td>AO₁</td>
<td>Chemically synthesized fatty acid hydrazide of Acid Oil.</td>
</tr>
<tr>
<td>OR₂</td>
<td>Chemically synthesized thiosemicarbazide.</td>
</tr>
<tr>
<td>AO₂</td>
<td>Chemically synthesized thiosemicarbazide.</td>
</tr>
<tr>
<td>OB₁</td>
<td>Chemically synthesized Schiff base by using benzaldehyde. 2-alkyl-N-benzylidenehydrazinecarbothioamide</td>
</tr>
<tr>
<td>OB₂</td>
<td>Chemically synthesized Schiff base by using vannilene. 2-alkyl-N-(4-hydroxy-3-methoxy benzylidene)hydrazinecarbothioamide</td>
</tr>
<tr>
<td>AB₁</td>
<td>Chemically synthesized Schiff base by using benzaldehyde. (2-alkyl-N-benzylidenehydrazinecarbothioamide).</td>
</tr>
<tr>
<td>AB₂</td>
<td>Chemically synthesized Schiff base by using vannilene. (2-alkyl-N-(4-hydroxy 3-methoxy benzylidene) Hydrazinecarbothioamide</td>
</tr>
<tr>
<td>AB₃</td>
<td>Chemically synthesized Schiff base by using P-hydroxybenzaldehyde (2-alkyl-N-(4-hydroxybenzylidene)hydrazinecarbothioamide)</td>
</tr>
<tr>
<td>AB₄</td>
<td>Chemically synthesized Schiff base by using furfural. (2-alkyl-N-(furan-2-ylmethylene)hydrazinecarbothioamide)</td>
</tr>
<tr>
<td>OB₃</td>
<td>Chemically synthesized Schiff base by using P-hydroxybenzaldehyde (2-alkyl-N-(4-hydroxybenzylidene)hydrazinecarbothioamide)</td>
</tr>
</tbody>
</table>
OB₄ Chemically synthesized Schiff base by using furfural.
(2-alkyl-N-(furan-2-ylmethylene)hydrazinecarbothioamide)

Figure 1: IR spectrum of OR₁

Figure 2: IR spectrum of AO₁
Figure 3: IR spectrum of OR₂

Figure 4: IR spectrum of AO₂
Figure 5: IR spectrum of OB₁

Figure 6: IR spectrum of OB₂
Figure 7: IR spectrum of AB₁

Figure 8: IR spectrum of AB₂
Figure 9: IR spectrum of AB$_3$

Figure 10: IR spectrum of AB$_4$
Figure 11: IR spectrum of OB$_3$

Figure 12: IR spectrum of OB$_4$
Figure 13: Anti bacterial activity of Schiff bases
4.5(A).5 Discussion

4.5.(A).5.1 FTIR analysis

OH group in IR Spectra of Schiff bases (fig.5-12) of oil showed the characteristic bands at around 3370 - 3450 cm\(^{-1}\). Bands at around 3180- 3250cm\(^{-1}\) for primary & secondary NH\(_2\) group are visible. A band at around 2849- 2919 cm\(^{-1}\) was observed for CH\(_3\), CH\(_2\), & CH stretching vibration. A band at around 1660- 1750 cm\(^{-1}\) was observed for Ketone (C=O) stretching vibration. A band at around 640 -722cm\(^{-1}\)was observed for Ar-CH-S bending vibration.

Since Schiff bases consist of one or more of aromatic group in their structure, they are expected to show some pharmacological properties which will vary depending upon the type of aldehydes used. Also, since the fatty thiosemicarbazides used in preparation of Schiff bases show some antibacterial and antifungal activities, Schiff bases made from them must show some of the properties exhibited by thiosemicarbazides.

4.5.(A).5.2 Microbial activities of Schiff bases

The results of antibacterial activities of Schiff bases given in Table 2, highlight following points:

The synthesized Schiff bases show mild activity against *Escherichia coli* while other bacteria which are selected for our study are not affected. Some photographs of antibacterial activity of Schiff bases are shown in fig.13.

The results of antifungal activities of Schiff bases given in Table 3, highlight that the synthesized Schiff bases are not active against selected fungi (*Aspergillus Niger and Candida Albicans*).

The various Schiff bases prepared from the fatty acid hydrazides of the various nontraditional oils like neem, karanja and
rice bran oil show good antibacterial and antifungal activities which are reported\textsuperscript{66}, but the Schiff bases prepared in the present study were made from ORSBE of soybean oil (which is an edible oil) and acid oil not containing any toxic non glyceride components and flavonoids, the mild to poor antibacterial and antifungal activities are possible and may also be due to the different R (alkyl) groups in oils. The R group present in the neem, karanja and rice bran oil may possess inherent biological activity which may be the reason behind the good biological activities shown by them, whereas the R group of ORSBE and acid oil may not possess the inherent biological activity, thus its Schiff bases show poor activity. Another reason for mild microbial activity may be due to the absence of the biologically active components in the oils used in the present study.
4.5(A).6 Conclusion

It can be concluded from present study that the Schiff bases prepared from the oil taken for study show poor to mild antibacterial activity against *Escherichia coli*. Further modification of Schiff bases by changing aldehyde or changing the oil may improve biological activity.
4.5 (B) Experimental

4.5(B).1 Preparation of Triazines derivatives

1. Preparation of methyl ester from oil

Methyl esters were prepared from oils by the same method as reported earlier in 4.5.1.1 (A).

2. Preparation of Fatty acid hydrazides

Fatty acid hydrazides were prepared from methyl esters by the same method as reported earlier in 4.5.1.2 (A).

3. Preparation of Triazines derivatives

A mixture of fatty acid hydrazide (2 mmol), diketone (2 mmol) and silica gel was ground in a pestle, NH$_4$OAc and Et$_3$N were added in catalytic amounts and the prepared mixture in an open beaker was subjected to microwave irradiation for the appropriate time (8 min for 3-alkyl-5,6-dimethyl-[1,2,4] triazine and 10 min for 3-alkyl-5,6-diphenyl-[1,2,4] triazine at 100% power). After complete conversion the mixture was extracted with petroleum ether and washed with water (3x50 ml). Then the solvent was evaporated in vacuum and the product was weighed.
Scheme: Chemically synthesized hydrazides and their Triazine derivatives

A = Fatty acid Hydrazide

B = 3- Alkyl-5, 6-Dimethyl-[1,2,4] triazines

C = 3- Alkyl-5,6- Diphenyl-[1,2,4] triazines

4.5(B).2 Analysis of Triazine derivatives

1. Determination of melting point

Melting points of triazine derivatives were determined by the same method as reported earlier in 4.5.2.1 (A). The melting points of derivatives are shown in Table 4.

2. Determination of nitrogen content by Kjeldahl’s Method

Nitrogen contents of triazine derivatives were determined by the same method as reported earlier in 4.5.2.2 (A). The nitrogen contents of derivatives are shown in Table 4.
3. Infrared Spectra of Triazine derivatives

Infrared spectra of derivatives were recorded by the same method as reported earlier in 4.5.2.3 (A). (Fig. 13-16)

4.5(B).3 Biological activity of Triazenes

1. Determination of anti-bacterial activity

Anti-bacterial activity of triazines was determined by the same method as reported earlier in 4.5.3.1 (A)

2. Determination of anti-fungal activity

Anti-fungal activity of triazines was determined by the same method as reported earlier in 4.5.3.2 (A)
4.5(B).4 Results

Table 4.

Analysis of hydrazides and their triazines

<table>
<thead>
<tr>
<th>Sample</th>
<th>M.Wt.</th>
<th>Melting point °C</th>
<th>% yield</th>
<th>Nitrogen Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Found</td>
</tr>
<tr>
<td>AO₁</td>
<td>242</td>
<td>87</td>
<td>80.3</td>
<td>10.37</td>
</tr>
<tr>
<td>OR₁</td>
<td>270</td>
<td>86</td>
<td>65.8</td>
<td>10.37</td>
</tr>
<tr>
<td>AT₁</td>
<td>345.57</td>
<td>*</td>
<td>59.5</td>
<td>12.16</td>
</tr>
<tr>
<td>OT₁</td>
<td>345.57</td>
<td>*</td>
<td>67.8</td>
<td>12.16</td>
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<td>AT₂</td>
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<td>469.70</td>
<td>*</td>
<td>75.7</td>
<td>8.95</td>
</tr>
</tbody>
</table>

* Melting point could not be determined due to paste like product.
Table 5.
Antibacterial activity of triazines

<table>
<thead>
<tr>
<th>Diameter of zone of inhibition</th>
<th>Test organism</th>
<th>AT1</th>
<th>OT1</th>
<th>AT2</th>
<th>OT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 mm</td>
<td>+ve control, Streptomycin (200 ppm) as +ve control, DMSO as –ve control.</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>10-15 mm</td>
<td>+ (++)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-20 mm</td>
<td>+ (+++)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-25 mm</td>
<td>+ (++++)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nature</th>
<th>Co</th>
<th>Gram Positivent</th>
<th>Bacillus subtilis</th>
<th>Bacillus cereus</th>
<th>Staphylococcus aureus</th>
<th>Micrococcus luteus</th>
<th>Escherichia coli</th>
<th>Salmonella typhi</th>
<th>Pseudomonas aeruginosa</th>
<th>Serratia Marcescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
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<tr>
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<td></td>
</tr>
</tbody>
</table>

Diameter of zone of inhibition <10 mm (+), 10-15 mm (++), 15-20 mm (+++), 20-25 mm (++++)

Concentration of tested compounds 200ppm, Streptomycin (200 ppm) used as +ve control, DMSO (Dimethyl Sulphoxide) used as –ve control.
Table 6.

**Antifungal activity of triazines**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>+ve control</th>
<th>-ve control</th>
<th>AT₁</th>
<th>OT₁</th>
<th>AT₂</th>
<th>OT₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Diameter of zone of inhibition <10 mm (+), 10-15 mm (++) , 15-20 mm (+++), 20-25(++++).

Concentration of tested compounds 200ppm, Imidil (Clotrimazol) 200 ppm used as +ve control, DMSO (Dimethyl Sulphoxide) used as −ve control.

**Derivative code**

- AO<sub>1</sub> = Fatty acid hydrazide of Acid oil
- OR<sub>1</sub> = Fatty acid hydrazide of ORSBE
- AT<sub>1</sub> = 3- Alkyl-5,6-dimethyl-[1,2,4] triazines of Acid oil
- OT<sub>1</sub> = 3- Alkyl-5,6-dimethyl-[1,2,4] triazines of ORSBE
- AT<sub>2</sub> = 3- Alkyl-5,6-diphenyl-[1,2,4] triazines of Acid oil
- OT<sub>2</sub> = 3- Alkyl-5,6-diphenyl-[1,2,4] triazines of ORSBE
Figure 14: IR spectrum of AT₁

Figure 15: IR spectrum of AT₂
Figure 16: IR spectrum of OT₁

Figure 17: IR spectrum of OT₂
Figure 18: Antibacterial activity of triazine derivative
4.5(B).5 Discussion

4.5(B).5.1 FTIR analysis

FT-IR spectrums of hydrazides (fig.1,2) show the characteristics absorption bands at 2850 and 2919 cm\(^{-1}\) due to C –H stretching of long alkyl chain. Absorption band at 1630 cm\(^{-1}\) is due to C=O stretching. C=O stretching frequency decreased due to adjacent N-H group. Absorption bands at 3320 cm\(^{-1}\) and 3291 cm\(^{-1}\) correspond to N-H group stretching, which is typical for primary amine and amide. Unsaturation of fatty hydrazides is seen at around 3040 cm\(^{-1}\) (C=C-H).

FT-IR spectrums of triazines (fig.14-17) show the characteristics absorption band at around 2852 cm\(^{-1}\) and 2922 cm\(^{-1}\) corresponding to C-H stretching of long alkyl chain. Absorption band around 1610 cm\(^{-1}\) corresponds to C=N stretching. Absence of 3320 cm\(^{-1}\) and 3291 cm\(^{-1}\) stretching clearly indicates the conversion of fatty hydrazide into triazine.

Since the fatty hydrazides made from by-products of oil processing industries used in the preparation of triazines show some antibacterial and antifungal activities\(^{65}\), triazines made from them are expected to show some of the properties exhibited by fatty acid hydrazides.

4.5.(B).5. 2 Microbial activities of triazines

The results of antibacterial activity of triazines (Table 5) highlight following points:

When triazines were tested for antibacterial activity, against Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Salmonella typhi, Psuedomonas aeruginosa and Serratia Marcescens, 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of acid oil (AT\(_1\)) showed mild bacterial growth retard at on against
Bacillus cereus and Escherichia coli, 3-Alkyl-5, 6-diphenyl-[1,2,4] triazines of acid oil (AT\textsubscript{2}) exhibited mild bacterial growth retardation against Bacillus subtilis and 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of ORSBE (OT\textsubscript{1}) showed mild bacterial growth retardation against Escherichia coli relative to streptomycin used as standard. Photograph of antibacterial activity of triazine derivatives are shown in fig.18.

The results of antifungal activity of triazines (Table 6) emphasize following points:
3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of acid oil (AT\textsubscript{1}), 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of ORSBE (OT\textsubscript{1}), 3-Alkyl-5, 6-diphenyl-[1,2,4] triazines of acid oil (AT\textsubscript{2}) and 3-Alkyl-5, 6-diphenyl-[1,2,4] triazines of ORSBE (OT\textsubscript{2}) showed poor antifungal activity against both the microorganisms.
4.5(B).6 Conclusion

On the basis of the present study it can be concluded that triazines made by microwave assisted synthesis from hydrazides of Acid oil and ORSBE, the two plentiful byproducts of oil processing industries, showed reasonable antibacterial and antifungal activities against some of the microorganisms used. A thorough screening of the microorganisms is however needed for future work.
4.6 References


55. BIS; 548 (Part III Analysis by liquid chromatography) methods of sampling and test for oil and fats, (Bureau of Indian standard, New Delhi) (1976).
73. BIS: 548. (Part -1), Methods of sampling and test for oils and fats (Bureau of Ind. standards, New Delhi) (1976).