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Enzymatic synthesis of nontraditional oils based hydrazides and microwave assisted synthesis of triazines and their evaluation as antimicrobial agents

S.D. Toliwal*, Kalpesh Jadav*, Bhumika Bhatt, Nidhi Verma**and Namita Jha****

**Department of Industrial Chemistry, Institute of Science and Technology for Advanced Studies and Research (ISTAR), Vallabh Vidyanagar, Gujarat, India*
*** B.R.D.School of BioScience, Vallabh Vidyanagar, Gujarat, India*

Abstract

Enzymatic hydrazides were made from nontraditional oils by a one step lipase catalysed 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.

Keywords: Nontraditional oils, Enzymatic hydrazides, Triazine, Antibacterial, Antifungal, Streptomycin, Imidil.

Introduction

Hydrazides have received a lot of attention due to their biological activity as tuberculostatic [1], antibacterial agent [2], antitumor agent [3] and anticancer agents [4]. Besides being utilizable for a wide range of pharmaceutical important derivatives, Hydrazides in view of their high reactivity are also important starting materials and intermediates in the synthesis of certain amides, aldehydes, and heterocyclic compounds that form acyl hydrazones [1]. Hydrazides also have been used for analytical chemistry as chelating agents [5].

The triazine is structurally a heterocyclic ring analogous to the six membered benzene but a three carbon compound replaced by nitrogens. Triazines synthesized from fatty acid hydrazides are a representative class of heterocyclic compounds with a wide variety of properties useful in medicinal and agricultural applications [6-9]. They have been associated with diverse pharmacological activities such as antihypertensive and inhibition of platelets [10], antileukemic [11], anti-inflammatory [12] and potent neuroprotective agents [13]. The 1,2,4 triazine moiety is a structural element in antimalarial [14], anticancer [15], antifungal [16], anticonvulsant [17], antibacterial [18] and antiviral [19] compounds. Certain compounds containing a 1,2,4 triazine nucleus have been reported to possess pesticidal [20],neuropharmacological [21], analgesic and antidepressant [22] properties. Some 1,2,4-triazine derivatives are used for the determination of metal ions and as dyes [10]. N-methyl derivatives of 1,2,4-triazines are the naturally occuring antibiotics fervenulin (planomycin), toxoflavin (xanthothricin) and reumycin.

Enzymatic Synthesis of hydrazides

The most widely used method to prepare hydrazides is to treat the corresponding esters with hydrazine monohydrate [23]. The reaction involving unreactive esters generally requires refluxing for a few hours in a basic condition and their for is hazardous and energy intensive 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 a mild reaction conditions, no by-products and the increased yield.

Microwave assisted synthesis of 3,5,6-trisubstituted 1,2,4 triazine

Two derivatives use of microwave irradiation is an established tool in organic synthesis for achieving better selectivity, rate enhancement and reduction of thermal degradation of byproducts [24-25]. Microwave assisted organic synthesis (MAOS) can facilitate the discovery of new reactions and reduce cycle time in optimization of reactions. Moreover it is an acknowledged quick alternative and green technology in synthetic organic chemistry that also provides easier work up procedures. However these procedures are practically limited as solvents in a microwave at elevated temperatures create high pressure which may cause explosion.

One of the advances to overcome this problem is inorganic solid supported organic synthesis which attracts attention because of enhanced selectivity, milder reaction conditions and associated ease of manipulation. It also provides an opportunity to work with open vessels and enhanced possibility for scaling up reactions [26-27].

The present work with a view to impart value addition targets plentiful nontraditional oils- neem, rice bran and karanja oil for making fatty hydrazides which can be further derivatized to obtain 3-alkyl-5,6-dimethyl-[1,2,4] triazine and 3-alkyl-5,6-dipenyl-[1,2,4] triazine were prepared in solvent free condition by microwave irradiation, new antibacterial and antifungal agents.

Up gradation and utilization of non traditional oils has been the subject of various investigative studies [28-39].

Materials and Methods

Oils were procured from Mahavir oil industries Ltd, Mahemdavad. Physico-chemical analysis of

neem, rice bran and karanja by standard BIS methods [40] gave, respectively : sp.gr$^{25\ 0C}$, 0.917, 0.918 and 0.921; acid value, 15.41,14.12 and 17.34; iodine value, 75.01,109.42 and 90.78; refractive index at 25 ^{0}C, 1.465, 1.462 and 1.458; and sponification value, 188.16,185.62 and 189.71. Fatty acid composition of oils (Table 1) was determined by gas liquid chromatography [41] (GLC) by Perkin Elmer Auto system XL gas chromatograph with flame ionization detector (FID). The capillary column BP-225 (moderate polar, 25m x 0.22mm x 0.25 microns) packed with 50% cynopropyl phenyl polysiloxane at 220^{0}C was used with nitrogen as carrier gas at flow rate 1 ml/min at an injector temperature of 250^{0}C. All other chemicals were of laboratory grade and were used without any modification.

Table 1. Fatty acid composition of oils

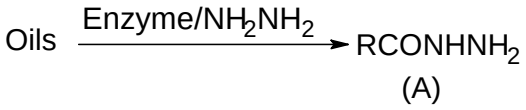
Sr. No.	Fatty Acid	Neem Oil	Rice Bran Oil	Karanja Oil
1.	Palmitic	16.96	18.5	10.95
2.	Stearic	16.39	2.5	6.57
3.	Oleic	49.31	43	57.3
4.	Linoleic	13.95	32	17.74
5.	Linolenic	1.85	2.2	2.85
6.	Arachidic	1.54	1.8	4.59

Preparation of Enzymatic fatty acid hydrazide

For the preparation of Enzymatic fatty acid hydrazide [42] the reaction was carried out by shaking 2.60 mmol of oils in hexane with hydrazine monohydrare which was neutralized by 5 M HCl in the presence of a chosen amount of a lipase in a 100 ml stoppered flask. The reaction mixture was shaken in a water bath at 200 rpm for 24 h at 40˚C.

Separation and purification of fatty acid hydrazides

Hot hexane was added to the reaction mixture to dissolve the product. The organic phase was then separated from the water phase using a separating funnel. To obtain the pure fatty hydrazide, the hexane phase was cooled (< 5˚C) and filtered and then recrystallized in hexane. The product was then dried in vacuum desiccator over phosphorous pentoxide. The dried product was weighed.



Scheme.1 Enzymatically synthesized hydrazides

R - Fatty alkyl chain

A- Enzymatic Fatty acid hydrazide

Preparation of microwave assisted 3,5,6-trisubstituted-1,2,4-triazines

Methyl esters from oils

Methyl esters from oils were prepared by acid catalyzed esterification method [43] 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 h 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 acids. The esters were purified by distillation under 4-5 mm Hg pressure.

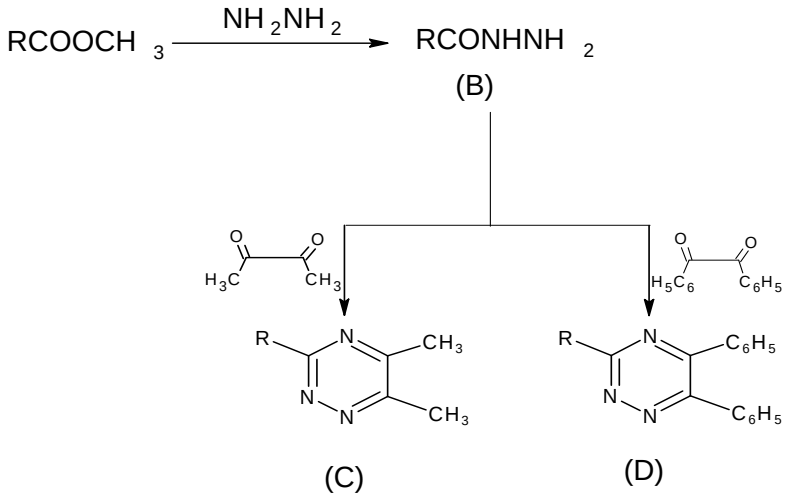
Fatty acid hydrazides

For the preparation of fatty acid hydrazides [44] a solution of fatty acid esters (0.1M) in ethanol (150 ml) was mixed with hydrazine hydrate (95%, 0.2 M) was added. The reaction mixture was refluxed for 3-4 h. It was cooled, and the solid separated was collected, washed and recrystallised from ethanol.

Triazines

For the preparation of triazines [45] a mixture of fatty acid hydrazide (2 mmol), diketone (2 mmol) and silica gel was ground in a pestle, ammonium acetate and ethylene tryamine were added in catalytic amounts and the prepared mixture in an open pyrex beaker was subjected to microwave irradiation for appropriate time (8 mins for 3-Alkyl-5, 6-dimethyl-[1,2,4] triazine and 10 mins for3-Alkyl-5, 6-diphenyl-[1,2,4] triazine). After complete conversion, the mixture was extracted with petroleum ether (3x50ml) and washed with water (3x50 ml). Then the solvent was evaporated in vacuum.

The preparation of fatty hydrazides and their triazines can be schematically shown as under:



Scheme.2 Fatty acid hydrazides and their triazines derivatives

R - Fatty alkyl chain
B - Fatty acid hydrazides
C - 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines
D - 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines

Characterization of Hydrazides and triazines

FTIR spectra of hydrazides (Figure 1 to 3) shows the characteristic absorption bands at 2850 & 2906 cm^{-1} due to C-H stretching of long alkyl chain. Absorption band at around 1746 cm^{-1} is due to C=O stretching. Absorption bands at 3320 cm^{-1} & 3220 cm^{-1} corresponds to N-H group stretching, which is typical for primary amine & amide. Additional characteristic absorption band at 1600-1630 cm^{-1} corresponds to C=O secondary amide & N-H stretching in primary amine. Unsaturation of fatty hydrazides is shown at 3010 – 3040 cm^{-1} (C –H alkenes; C = C-H) and 1600 cm^{-1} (alkenes; C= C).

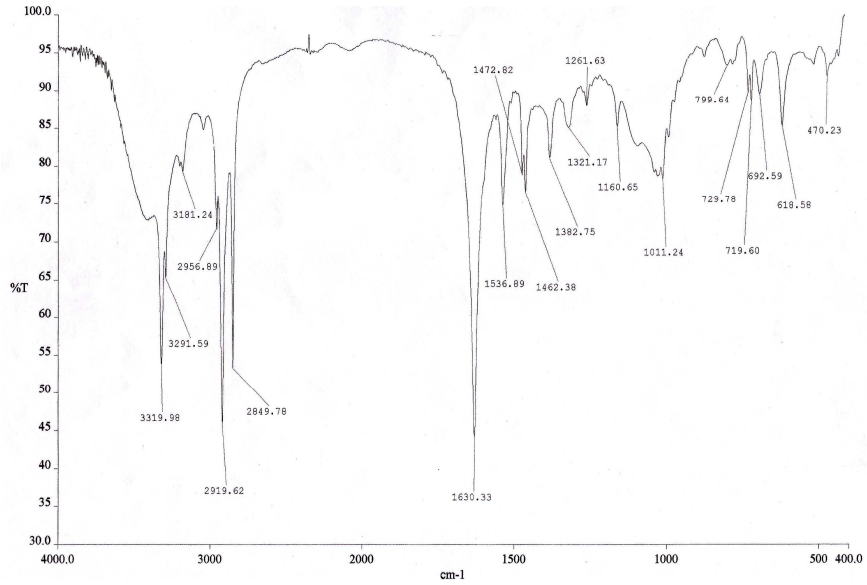


Figure 1 IR spectrum of DN_1

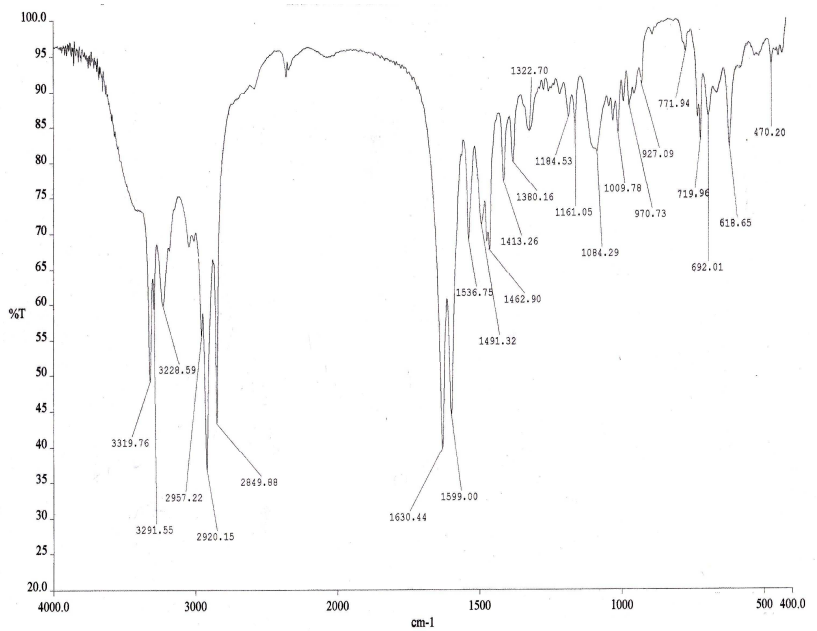


Figure 2 IR spectrum of DR_1

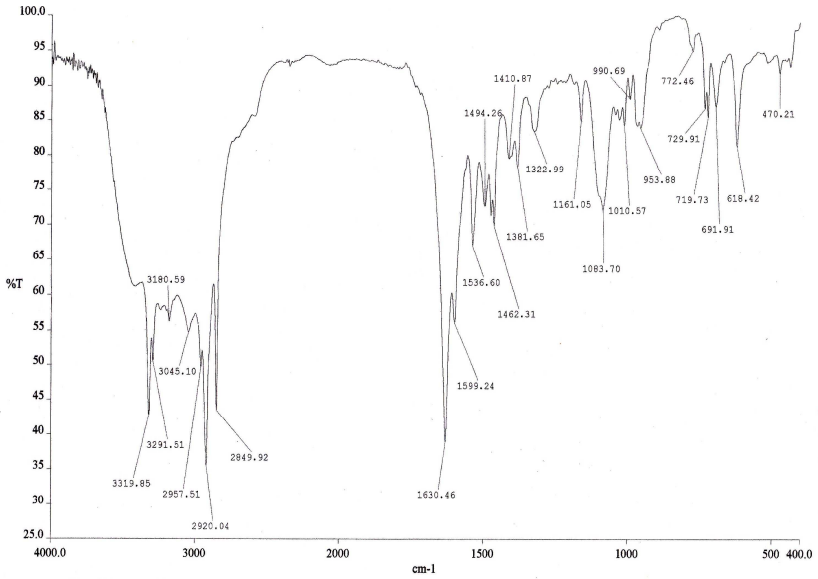


Figure 3 IR spectrum of DK_1

FT-IR spectra of triazines (Figure 4 to 9) shows the characteristic absorption band at 2850cm^{-1} and 2906 cm^{-1} corresponding to C-H stretching of long alkyl chain. Absorption band around 1620 cm^{-1} corresponds to C=N stretching. Absence of 3320 cm^{-1} and 3220 cm^{-1} stretching, clearly indicates the conversion of fatty hydrazides into triazines.

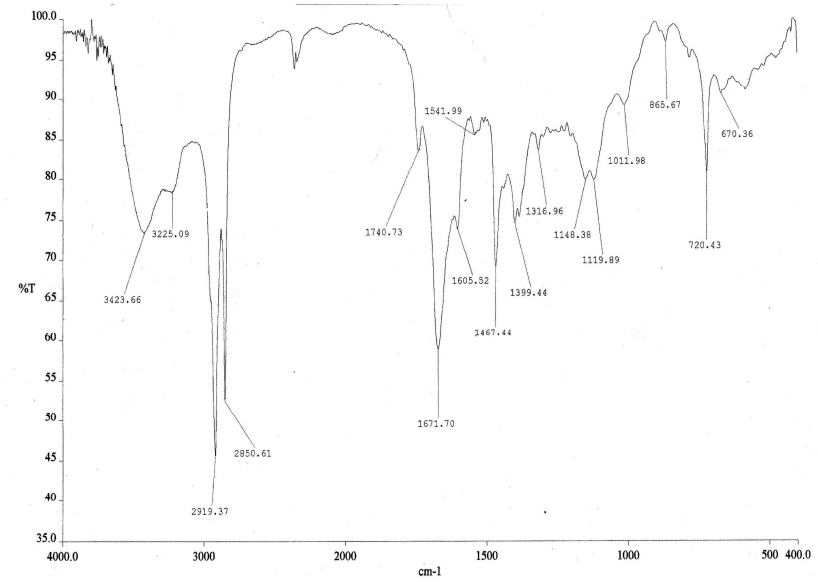


Figure 4 IR spectrum of CN_1

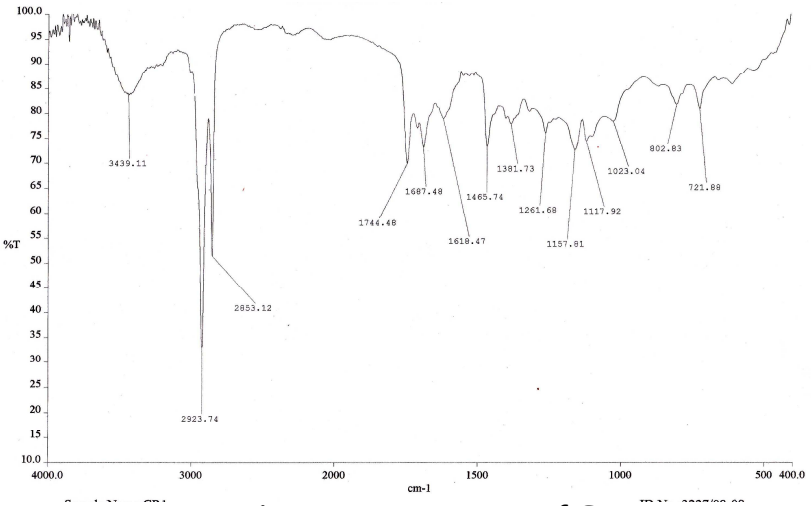


Figure 5 IR spectrum of CR_1

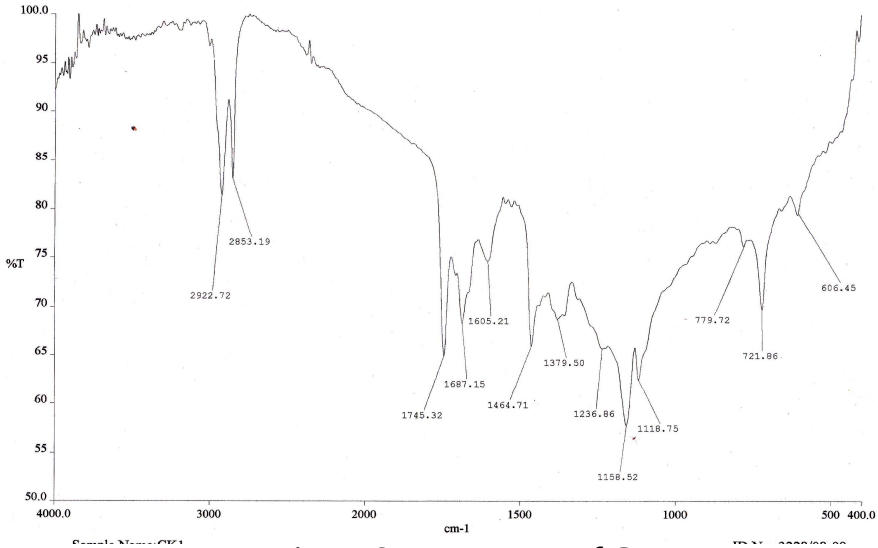


Figure 6 IR spectrum of CK_1

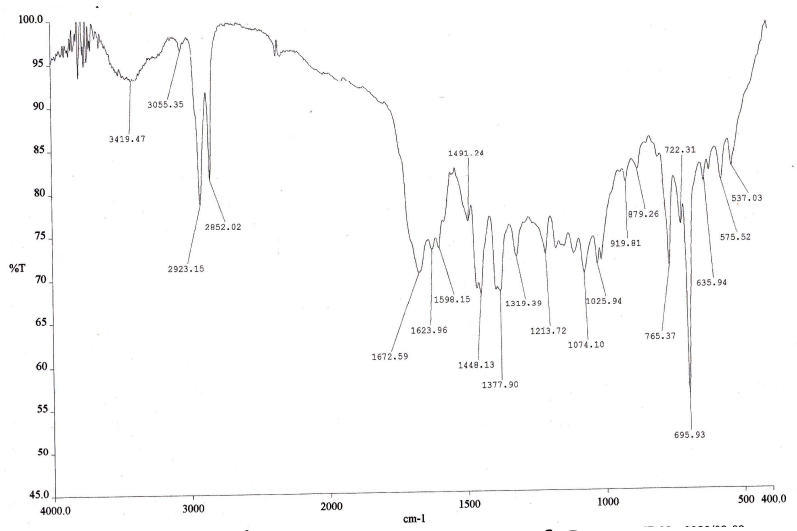


Figure 7 IR spectrum of CN_2

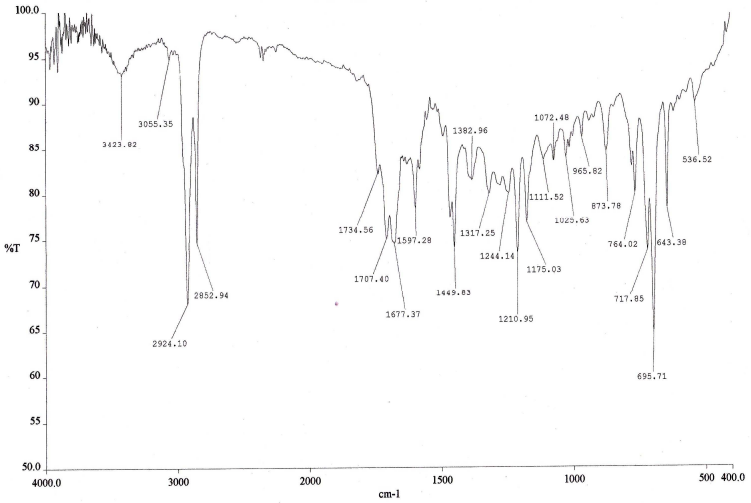


Figure 8 IR spectrum of CR_2

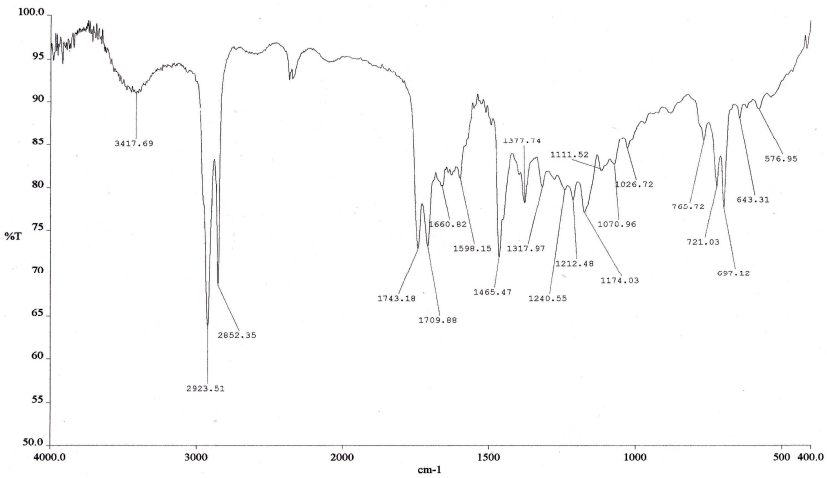


Figure 9 IR spectrum of CK_2

Analysis of derivatives

The synthesized enzymatic hydrazides and triazines were evaluated for melting point [46] and nitrogen content [47] by standard BIS methods. Their percent yield was also determined.

The enzymatic hydrazides and triazine were tested for antimicrobial activity by agar-agar cup method [48]. Streptomycin and imidil were used as standard antibacterial and antifungal agents respectively.

Results and Discussion

The melting points, percent yield and nitrogen contents for all the hydrazides and triazines are reported in Table 2. It can be seen that found and calculated percent nitrogen contents both are quite close and match fairly well.

Table 2. Physico-chemical properties of enzymatic hydrazides and triazines

Sr.No	Sample	Molecular Weight	Melting point ^{0}C	% Yield	Nitrogen Content (%)	
					Calculated	Found
1	DN_1	296.49	92	97	9.45	8.74
2	DR_1	296.49	95	98	9.45	9.30
3	DK_1	296.49	89	96	9.45	9.24
4	CN_1	345.57	*	44	12.16	11.23
5	CR_1	345.57	*	63	12.16	9.47
6	CK_1	345.57	*	72	12.16	10.58
7	CN_2	469.70	*	83	8.95	6.10
8	CR_2	469.70	*	67	8.95	6.95
9	CK_2	469.70	*	68	8.95	8.53

* = Melting point could not be determined due to paste-like consistency of the products.

Derivative Code

DN_1 = Enzymaticaly (lipase) synthesized fatty acid hydrazide of neem oil
DR_1 = Enzymaticaly (lipase) synthesized fatty acid hydrazide of rice bran oil
DK_1 = Enzymaticaly (lipase) synthesized fatty acid hydrazide of karanja oil
CN_1 = 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of neem oil
CR_1 = 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of rice bran oil
CK_1 = 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of karanja oil
CN_2 = 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of neem oil
CR_2 = 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of rice bran oil
CK_2 = 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of karanja oil

Biological Evaluation of Enzymatic Hydrazides and triazines

The results of antimicrobial activities of enzymatic hydrazides and triazines (Table 3) highlight following points:

Table 3. Antimicrobial activity of neem, rice bran and karanja oil enzymatic hydrazides and triazines

Organism	DN_1	DR_1	DK_1	CN_1	CR_1	CK_1	CN_2	CR_2	CK_2	+Ve Control	-Ve Control
Bacillus subtilis	++	+++	++	-	-	++	-	+	+	++	--
Bacillus cereus	+	++	+	-	-	+	-	++	+	+++	--
Staphylococcus aureus	++	++	+++	+	-	+	-	+	+	+++	--
Staphylococcus epidermidis	+	+	++	+	-	+	-	+	+	++	--
Micrococcus luteus	++	++++	+	-	++	+	-	-	+++	+++	--
Enterococcus faecalis	++	+++	++	+++	++	++	+++	+++	++	+++	--
Escherichia coli	+++	+++	+	+	+++	+++	-	+	+	+++	--

Salmonella typhii	+	++	++	-	-	+	-	++	++	+++	--
Salmonella paratyphii	+++	+++	++	-	++	+	-	+	-	+++	--
Psuedomonas aeruginosa	++	++++	+	+	-	+	-	+	+	+++	--
Serratia Marcescens	++	+	++	-	++	+	-	+	+	+++	--
Klebsiella pnuemoniae	++	++	+++	-	+++	+	-	+	+	+++	--
Aspergillus niger	+	++	-	+	-	-	++	+	-	++	--
Candida albicans	+	++	+	-	-	-	-	+	++	++	--

No inhibition (-), 0-10mm (+), 11-20mm (++), 21-30mm (+++),>30mm (++++)
+Ve control (Streptomycin and Imidil), –Ve control (Dimethyl sulphuroxid)

When enzymatic hydrazides and triazines were tested for antibacterial activity against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Enterococus faecalis, Escherichia coli, Salmonella typhii, Salmonella paratyphii, Psuedomonas aeruginosa, Serratia Marcescens,* and *Klebsiella pnuemoniae* neem oil hydrazides (DN_1)showed better bacterial growth against *Escherichia coli* and *Salmonella paratyphii*, rice bran oil hydrazides (DR_1) exhibited excellent bacterial growth against *Micrococcus luteus* and *Psuedomonas aeruginosa* and karanja oil hydrazides (DK_1) showed better bacterial growth against *Staphylococcus epidermidis* and *Klebsiella pnuemoniae*. 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of neem oil (CN_1) showed better bacterial growth against *Enterococus faecalis,* 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of rice bran oil (CR_1) exhibited better bacterial growth against *Escherichia coli* and *Klebsiella pnuemoniae,* 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of karanja oil (CK_1) showed better bacterial growth against *Escherichia coli,* 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of neem oil (CN_2) and 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of rice bran oil (CR_2) exhibited better bacterial growth against *Enterococus faecalis,* 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of karanja oil (CK_2) showed better bacterial growth against *Micrococcus luteu.*

Enzymatic hydrazides and triazines tested for antifungal activity against *Aspergillus niger* and *Candida albicans* neem oil hydrazides (DN_1) showed mild fungal growth against both the organisms, rice bran oil hydrazide (DR_1) exhibited good fungal growth against both the microorganisms and karanja oil hydrazide (DK_1) showed mild fungal growth against *Candida albicans.* 3-Alkyl-5, 6-dimethyl-[1,2,4] triazines of neem oil (CN_1) and 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of neem oil (CN_2) showed mild and good fungal growth against *Aspergillus niger* respectively, 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of rice bran oil (CR_2)showed mild fungal growth against both the microorganisms and 3-Alkyl-5, 6-diphenyl-[1,2,4]triazines of karanja oil (CK_2) showed good fungal growth against *Candida albicans.*

Conclusion

It can be concluded that some fatty hydrazides and their triazines made from neem, rice bran and karanja oil can be used as antibacterial agent against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Enterococus faecalis, Escherichia coli, Salmonella typhii, Salmonella paratyphii, Psuedomonas aeruginosa, Serratia Marcescen,* and as antifungal agent against *Aspergillus niger* and *Candida albicans.*

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