Synthesis and biological evaluation of phenyl hydrazides and their derivatives from Rice bran oil

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Abstract

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 were synthesized 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.

Keywords: Rice bran oil, Phenyl hydrazides, Oxadiazole, Triazole, Antibacterial, Antifungal, Streptomycin, Imidil.

Introduction

Hydrazides are important starting materials for a wide range of derivatives utilizable in pharmaceutical products, corrosion inhibitors and surfactants. Their derivatives such as thiosemicarbazides possess useful pharmacological properties. Several triazole derivatives obtainable from hydrazides exhibit antibacterial activities [1] in addition to fungicidal[2], insecticidal[3-4] and herbicidal[5] activities. Some derivatives of fatty hydrazides such as phenyl thiosemicarbazides, 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiols, 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazoles, (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amines have gained attention and importance owing to their biological properties like bacteriostatic, insecticidal and antifungal activities.

Upgradation and utilization of non-traditional oils has been the subject of various investigative studies[6-15]. The present work targets neem oil, an important non traditional oil for preparation of phenyl hydrazides and their derivatives for their evaluation as antibacterial and antifungal agents.

Results and Discussion

The physico chemical characteristics and fatty acid composition of the Rice bran oil are shown in Table-1 and Table-2 respectively.
The result and analysis of all the phenyl hydrazides and their derivatives are presented in Table-3. It can be observed that upon cyclization to 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole, Phenyl thiosemicarbazide, 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol and (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine the melting points are increased and are higher than that of the original phenyl hydrazide of Rice bran oil from which they are derived.

The melting point increase for various phenyl hydrazide derivatives were observed in the following order: -

5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol > Phenyl thiosemicarbazide > (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine

This result is in agreement with the results obtained by Badami R. C. et al[17] and Daulatabad C. D. et al[16]for corresponding fatty derivatives. The obtained percent yield for Rice bran oil phenyl thiosemicarbazide is 82.3% and matches fairly with percent yield reported for aryl thiosemicarbazide by Badani R. C. et al[17] where as the obtained percent yield for Rice bran oil phenyl hydrazides derivatives 5-alkyl-4 phenyl-4H-(1, 2, 4) triazol-3-thiol is 79.3% , (5-alkyl-(1, 3, 4)-oxadiazol-2-yl)-phenyl amine is 88.2% and are quite close to percent yield reported for fatty (oleic and linoleic) hydrazides derivatives 2-alkyl-5-aryl amino-1, 3, 4-oxadiazole ,3-alkyl 4 aryl-5-mercapto-1, 2, 4, 4H triazole by Daulatabad C. D. et al[16]. The theoretical and observed nitrogen contents for Rice bran oil phenyl hydrazides and their derivatives are quite close in agreement.

Biological Evaluation of Phenyl hydrazides and their Derivatives

The results of antibacterial activity of phenyl hydrazides and their derivatives shown in Table-4 highlight following points:
Phenyl hydrazides and 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole of rice bran oil extremely good (75-100%) bacterial growth retardation against *Escherichia Coli* where as poor bacterial growth retardation against *Bacillus Subtilis*. 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol showed good (50-75%) bacterial growth inhibition against *Escherichia Coli* where as poor bacterial growth retardation against *Bacillus Subtilis* and (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine of rice bran oil exhibited excellent (More than 100%) bacterial growth retardation against *Escherichia Coli* where as poor bacterial growth inhibition against *Bacillus Subtilis*. Phenyl thiosemicarbazide showed poor antibacterial activity against both the microorganisms. The results of antifungal activity of phenyl hydrazides and their derivatives shown in Table-5 emphsize following points:

Phenyl hydrazides, 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole, Phenyl thiosemicarbazide, 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol and (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine of Rice bran oil showed poor antifungal activity against both the microorganisms.

Materials and Methods

Rice bran oil was procured from Mahavir oil industries Ltd, Mahemdavad. Physico-chemical analysis of rice bran by standard BIS methods[18] (Table-1). Fatty acid composition of oils (Table-2) was determined by gas liquid chromatography[19] (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 was used at 220^0C with nitrogen as carrier gas at flow rate 1 ml/min at an injector temperature of 250^0C. All other chemicals were of laboratory grade and were used without any modification.

Table 1. Physico-chemical Characteristics of Rice bran oil

Characteristics	Rice bran oil
Acid Value (mg KOH/gm)	14.12
Sponification Value (mg KOH/gm)	185.62
Iodine Value	109.42
Specific gravity at 25^0 C	0.918
Refractive Index at 25^0 C	1.462

Table 2. Fatty Acid Composition of Rice bran oil

Fatty Acid	Percentage
Palmitic Acid	18.50
Stearic Acid	2.50
Oleic Acid	43.00
Linoleic Acid	32.00
Linolenic Acid	2.20
Arachidic Acid	1.80

Preparation of mixed fatty acids from oil

Mixed fatty acids from oil were prepared by Sponification method in which 100 gm oil was taken in 500 ml round bottom flask and 30% alcoholic NaOH was added. The contents were refluxed for 3 hours on water bath. At the end of reaction, the excess alcohol was distilled off and soap was dissolved in hot water. Then the fatty acids were liberated by acidifying the soap solution with 1:1 H_2SO_4 (added till development of red colour in methyl red), and washed and dried over anhydrous sodium sulfate.

Preparation of Phenyl hydrazides

Fatty acids (1.0g) were dissolved in 2 ml of phenyl hydrazine and the solution was boiled gently for 20 minutes. Upon cooling the solid product precipitated out in good yield by the addition of petroleum ether (40-60 ^{o}C). The precipitate was filtered off with suction and washed with small quantity of petroleum ether until the crystals were completely white. These crystals were recrystallised from alcohol or alcohol-water mixture[20].

Preparation of Phenyl thiosemicarbazide

To a solution of a phenyl hydrazide (0.02 M) in methanol (50 ml) a solution of potassium thiocynate (0.03 M) and Conc. 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 recrystallised from ethanol[21].

Preparation of 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol

To a solution of phenyl thiosemicarbazide (0.01 M) in 15 ml of ethanol, a solution of potassium hydroxide (20 ml, 10%) was added and the mixture was refluxed immediately for 7-8 hours on steam bath. It was cooled and acidified with dilute hydrochloric acid at pH 5-6. Resulting solid was filtered, dried and recrystallised from ethanol[22].

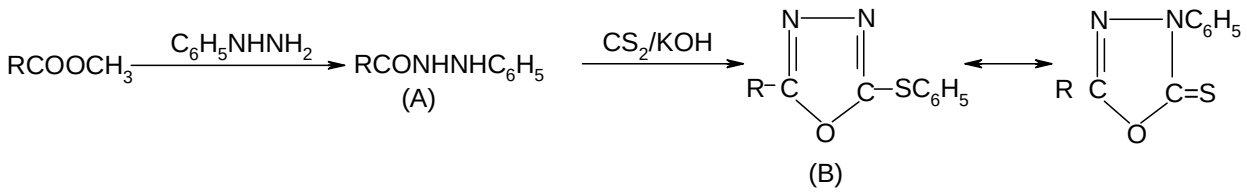
Preparation of (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine

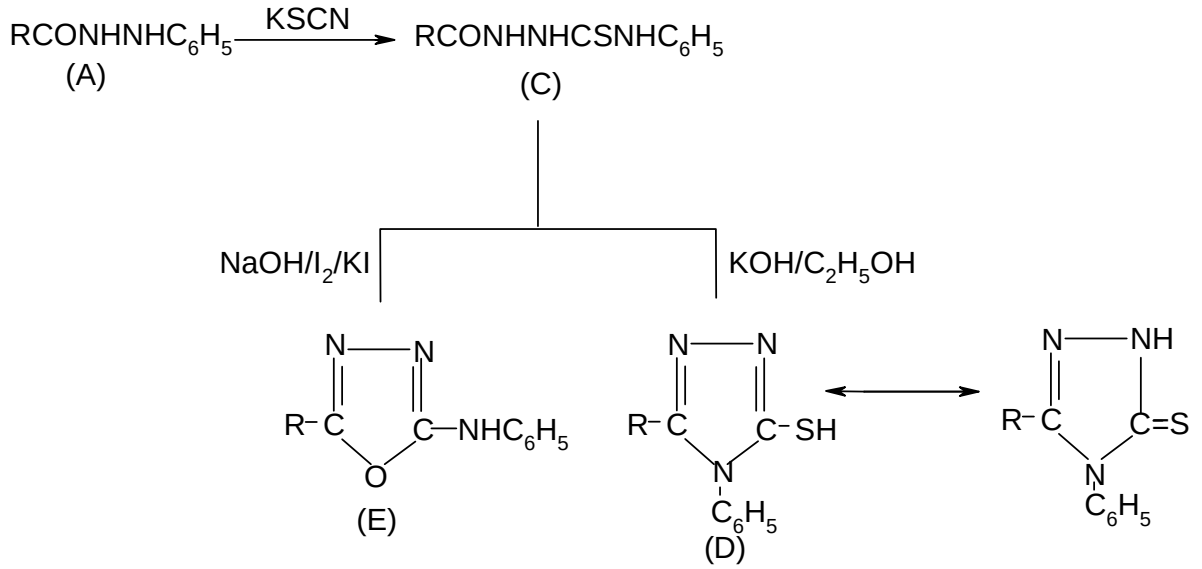
To a solution of phenyl thiosemicarbazide (0.01 M) in 15 ml of ethanol, a solution of sodium hydroxide (5 ml, 5N) was added with cooling and stirring. To this clear solution, a solution of I_2/KI was added till permanent tinge of iodine persisted at room temperature. The mixture was immediately refluxed and more I_2/KI was added till permanent tinge was obtained. It was cooled and poured in to ice cold water, the solid that separated was collected by filtration. It was washed with water and with dilute thiosulfate solution and again with water. It was dried and recrystallised from absolute ethanol[23].

Preparation of 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole

To a solution of a phenyl hydrazide (0.01 M) in 10 ml of ethanol, a solution of carbon disulfide (2 gm) in 3 ml of water and 1 gm of potassium hydroxide was added, and the mixture was refluxed for 7-8 hours until evolution of H_2S ceased. This was cooled and acidified with dilute hydrochloric acid. The solid that separated was collected, filtered, washed with water, dried and recrystallised from ethanol[24].

The preparation of phenyl hydrazides and their phenyl derivatives are schematically shown in Scheme. A.





Scheme. A Preparation of phenyl hydrazides and their phenyl derivatives

R- Fatty alkyl chain
A- Phenyl hydrazides
B- 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole
C- Phenyl thiosemicarbazide
D- 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol
E- (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine

Characterization of Phenyl hydrazides and their derivatives

The infrared spectra of phenyl hydrazides and their derivatives were recorded by FTIR- 8201 PC (Shimadzu) infrared spectrophotometer in KBr (Fig 1 to 5).

Spectral analysis of triazoles and oxadiazoles

Sample code	Functional Group	Observed Frequency in cm^{-1}
PR_1	N—H	3320
		3220
	C—H	3011
	—CH_3,>CH_2,CH— (Long chain alkyl)	2918
		2850
	C=O	1665
	C=H	3294
		3217

PR_2	—CH_3,>CH_2,CH— (Long chain alkyl)	2920
		2850
	=C—O—C=	1242
PR_3	C=H	3293
	N—H	3285
	—CH_3,>CH_2,CH— (Long chain alkyl)	2919
		2850
	C=N	1600
	C=S	1243
PR_4	N—H	3293
	—CH_3,>CH_2,CH— (Long chain alkyl)	2918
		2850
	C=N	1637
	C—N	1247
	C=S	1096
PR_5	N—H	3294
		3225
	C—H (Aromatic)	3030
	—CH_3,>CH_2,CH— (Long chain alkyl	2918
		2850
	C=N	1665
	=C—O—C=	1244

Analysis of derivatives

The synthesized compounds were evaluated for melting point[25] and nitrogen content[26] by standard BIS methods. Their percent yield was also determined (Table-3). The Phenyl hydrazides and their derivatives were tested for anti bacterial activity against *Bacillus Subtilis* and *Escherichia coli* (Table-4) and antifungal activity against Aspergillus niger and Candida albicam (Table-5) by agar-agar cup method. Streptomycin and immidil were used as standard antibacterial and antifungal agents respectively.

Table 3. Physico-chemical properties of phenyl hydrazides and their derivatives

Sample code	M. P ^{0}C	Yield %	Nitrogen Content (%)	
			Calculated	Found
PR_1	76	81.0	7.52	6.28
PR_2	69	76.1	6.76	7.98
PR_3	82	82.3	9.73	11.80
PR_4	83	79.3	10.16	9.04
PR_5	81	88.2	10.57	10.23

PR_1 = Phenyl hydrazides, PR_2 = 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole, PR_3 = Phenyl thiosemicarbazide, PR_4 = 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol, PR_5 = (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine

Table 4. Antibacterial activities of Rice bran oil phenyl hydrazides and their derivatives

	Bacillus subtilis				***Escherichia coli***			
Sample code	Zone standard 200ppm (mm)	Zone sample 200ppm (mm)	Control	growth %	Zone standard 200ppm (mm)	Zone sample 200ppm (mm)	Control	growth %
PR_1	21	-ve	---	---	21	19	---	++++
PR_2	22	-ve	---	---	22	19	---	++++
PR_3	22	-ve	---	---	22	-ve	---	---
PR_4	21	-ve	---	---	22	16	---	+++
PR_5	21	-ve	---	---	21	22	---	+++++

+ = 0-25 %, ++ = 25 – 50 %, +++ = 50 - 75 %, ++++ = 75 – 100 %, +++++ = More than 100%

Table 5. Antifungal activities of Rice bran oil phenyl hydrazides and their derivatives

	Aspergillus niger				***Candida albicans***			
Sample code	Zone standard 200ppm (mm)	Zone sample 200ppm (mm)	Control	growth %	Zone standard 200ppm (mm)	Zone sample 200ppm (mm)	Control	growth %
PR_1	22	-ve	---	---	22	-ve	---	---
PR_2	21	-ve	---	---	21	-ve	---	---
PR_3	20	-ve	---	---	23	-ve	---	---
PR_4	19	-ve	---	---	19	-ve	---	---
PR_5	23	-ve	---	---	20	-ve	---	---

+ = 0-25 %, ++ = 25 – 50 %, +++ = 50 - 75 %, ++++ = 75 – 100 %, +++++ = More than 100%.

Conclusion

Rice bran oil phenyl hydrazide derivatives such as 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol can be used as antibacterial agent against *Escherichia Coli* due to its good antibacterial activity relative to Streptomycin used as standard. Phenyl hydrazides and 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole can be used as antibacterial agent against *Escherichia Coli* due to their better antibacterial activity relative to Streptomycin used as standard and (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine can be used as antibacterial agent against *Escherichia Coli* due to its excellent antibacterial activity relative to Streptomycin used as standard.

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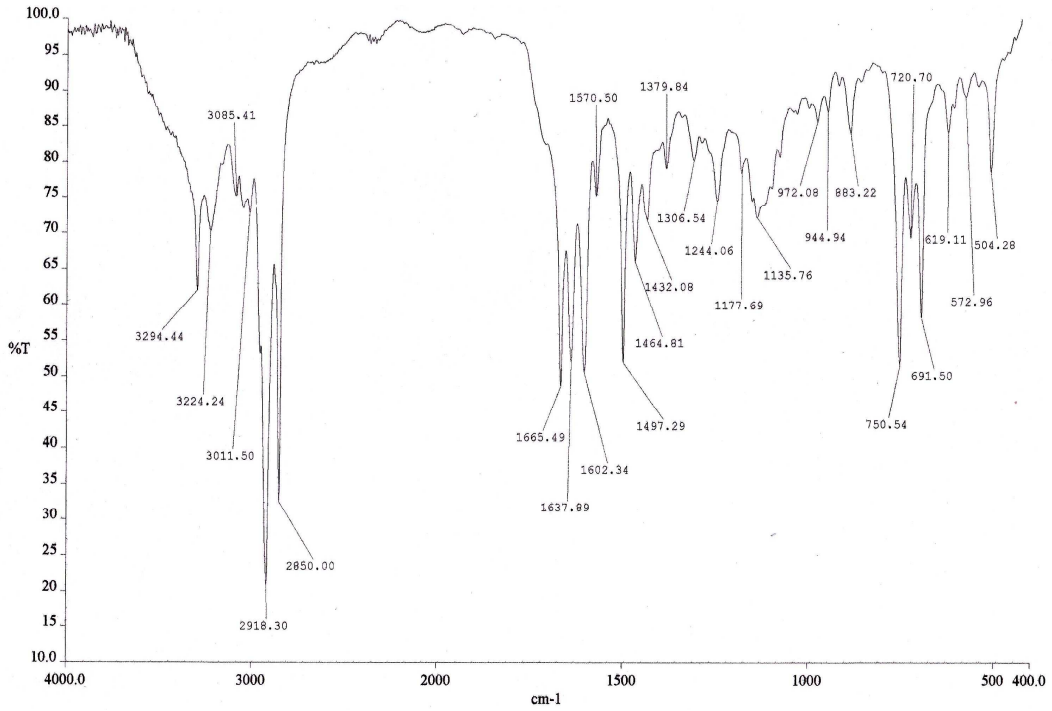

Fig. 1 IR spectrum of Phenyl hydrazides of rice bran oil

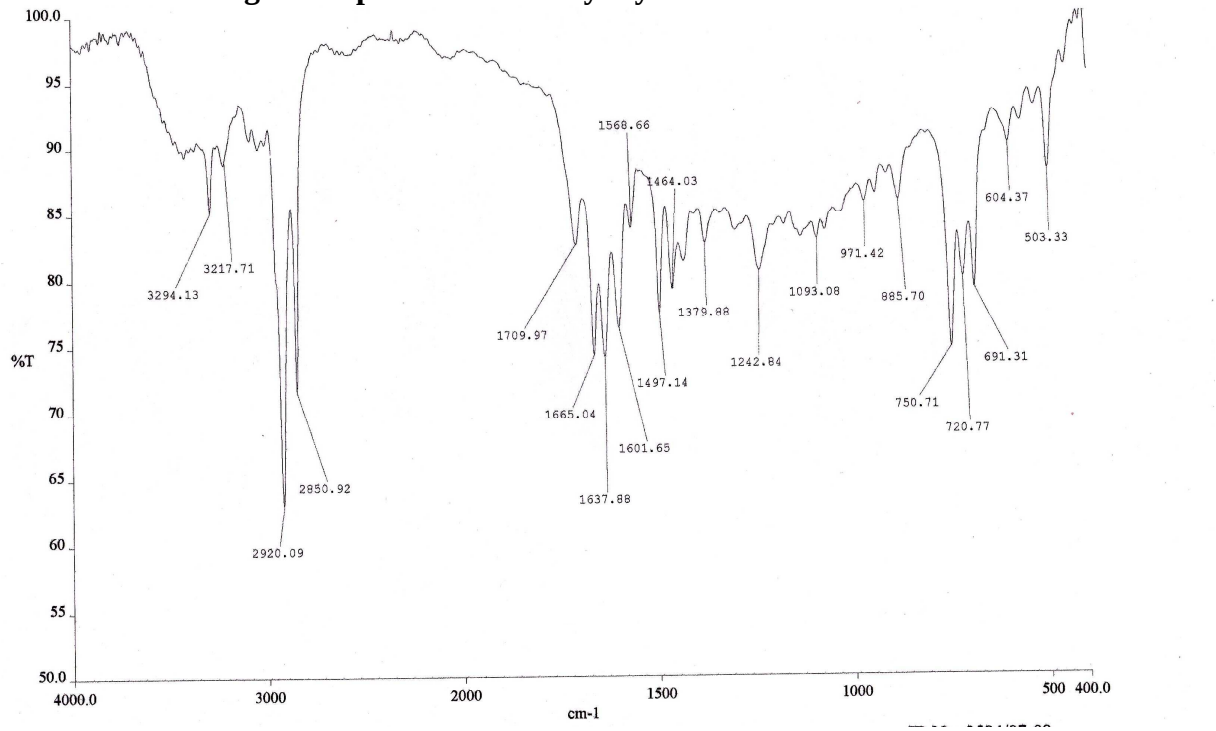

Fig. 2 IR spectrum of 2-alkyl-5-phenylsulfanyl-[1,3,4]oxadiazole of rice bran oil

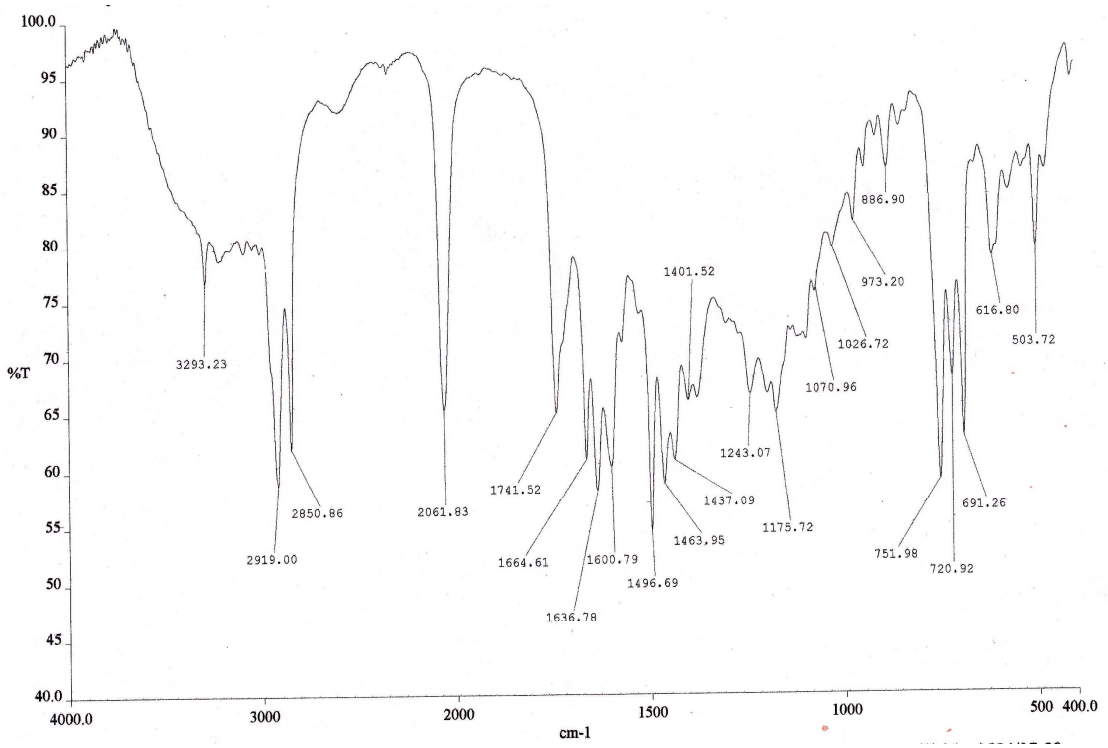

Fig. 3 IR spectrum of Phenyl thiosemicarbazide of rice bran oil

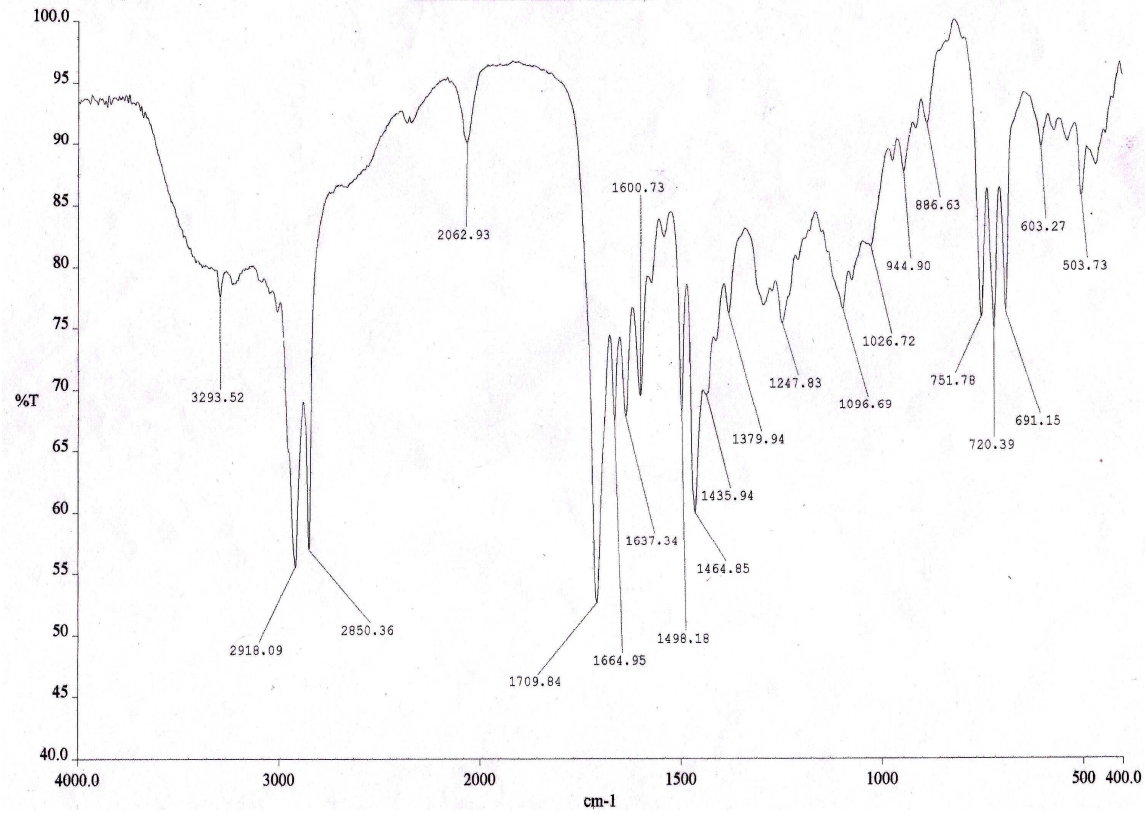

Fig. 4 IR spectrum of 5-alkyl-4 phenyl-4H-[1,2,4]triazole-3-thiol of rice bran oil

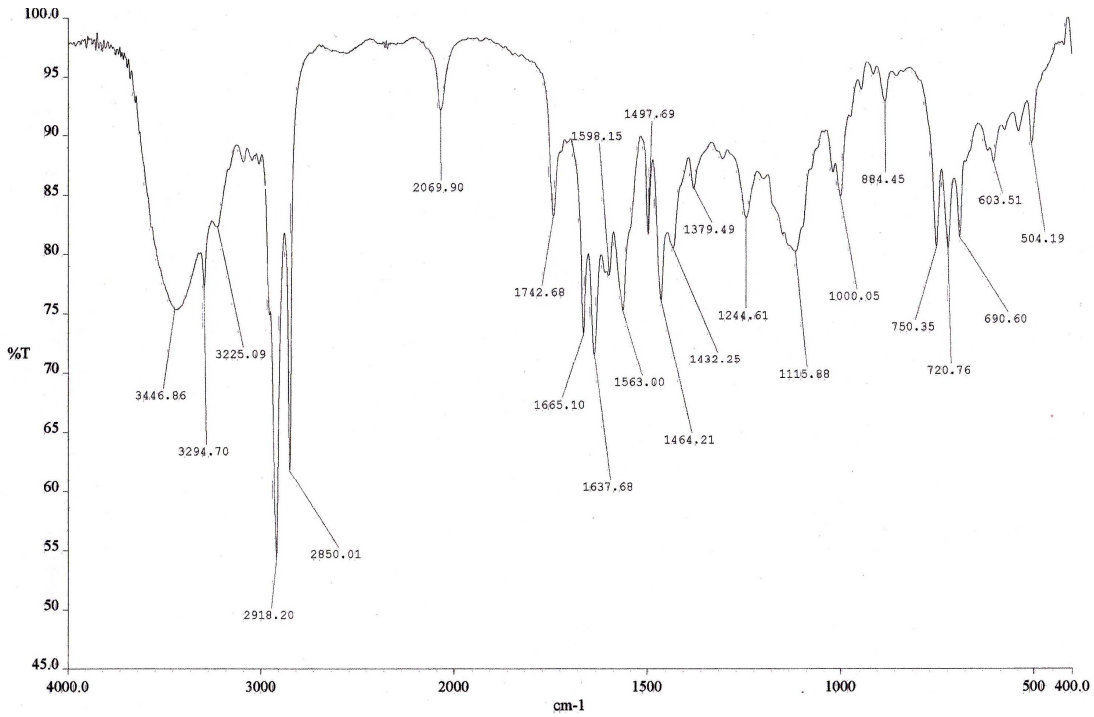

Fig. 5 IR spectrum of (5-Alkyl-[1, 3, 4]-oxadiazol-2-yl)-Phenyl amine of rice bran oil