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VIEWS

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Preconcentration and Solid Phase Extraction Method for the Determination of Co, Cu, Ni, Zn, and Cd in Environmental Samples Using Activated Carbon by FAAS

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ABSTRACT

4-(1-(2-hydroxyphenyl) ethylene amino)-2-hydroxy benzoic acid (HPEAHBA) was Synthesized for Solid Phase extraction (SPE) to the determination of Co, Cu, Ni, Zn and Cd in environmental samples by flame atomic absorption spectrometry (FAAS). These metals were sorbed as HPEAHBA complexes on activated carbon (AC) at the pH range of 5.0±0.2 and eluted with 6ml of 1M HNO_3 in acetone. The effect of sample volume, eluent volume and recovery has been investigated to enhance the sensitivity and selectivity of proposed method. The effect of interferences on the sorption of metal ions was studied. The concentration of the metal ions detected after pre-concentration was agreement with the added amount. The detection limits for the metals studied were in the range of 0.75-3.82ìgml^{-1}. The proposed system produced satisfactory results for the determination of Co, Cu, Ni, Zn and Cd in environmental samples.

Keywords: 4-(1-(2-hydroxyphenyl) ethylideneamino)-2-hydroxy benzoic acid (HPEAHBA); Solid Phase extraction (SPE); activated carbon (AC); environmental samples. ; Flame atomic absorption spectrometry (FAAS).

Introduction

Nowadays determination of trace metals in environmental samples are essential, because of these metals has been used in various industries. Various techniques have been reported for the determination of trace metals in environmental samples. Flame atomic absorption spectrometry (FAAS) has widely used for determination of trace metal ions. However, direct determination of metal ions at trace levels by FAAS is limited due to their low concentrations and matrix interferences [1]. In trace analysis, therefore, Pre-concentration leads to simplify trace metal determination. Several methods of pre-concentration include solvent extraction [2, 3] adsorption [4, 5], membrane extraction [6], co-precipitation [7-9], ion-exchange [10, 11]. But, Solid Phase extraction (SPE) is multi element pre-concentration method with simplicity, rapidity and ability to attain a high concentration factor. Activated carbon has been widely used for many purposes due to its ability [12-17], to adsorb organic compounds and organic metal complexes. Enrichment of trace metals using activated carbon has been carried out with very high pre-concentration factors in different matrices [18-28]. The standard method of determination of trace metals in environmental samples involves the use of ammonium pyrrolidine dithiocarbamate for complex formation, followed by extraction of the metal complex with methyl isobutyl ketone [29] and subsequent determined by Flame atomic absorption spectrometry. The above mentioned techniques require large amount of solvents and more time consuming.

Hence, there is a need to develop simple, sensitive reagent that requires less solvent pre-concentration method for the determination of metal ions in various environmental matrices. In the present study, 4-(1-(2-hydroxyphenyl) ethylene amino)-2-hydroxy benzoic acid (HPEAHBA) was synthesized and impregnated onto activated carbon for the Preconcentration of Co, Cu, Ni, Zn and Cd in environmental samples. The metals determination was performed by FAAS

Experimental

Apparatus

Flame atomic absorption spectrometer (Perkin-Elmer

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Model Analyst100) was used to determine metal concentrations using an air\acetylene flame. The instrumental parameters were those recommended by the manufacturer were represented in Table 1. The SPE was performed using 25ml polythene tubes and frits. A digital pH meter (Elico Li-129 Model) was used for all pH measurements

Reagents

All reagents and solvents of analytical grade were used without further purification. Double distilled water has been used for all reagent preparation. Working standard solution of Co, Cu, Ni, Zn and Cd (Merck Chemicals, Mumbai, India) were prepared by stepwise dilution of 1.0 μgmL^{-1} . Sodium acetate buffer solution was prepared by adding an appropriate amount of acetic acid to sodium acetate solution until pH 5.0 was attained.

Synthesis of 4-(1-(2-hydroxyphenyl) ethyleneamino)-2-hydroxybenzoic acid (HPEAHBA)

0.01 moles of 1-(2-hydroxyphenyl) ethanone dissolved in methanol was added to 0.01 moles of 4-amino-2-hydrxybenzoic acid in methanol and refluxed for 4 hours and kept freeze in overnight. A reddish brown solid was formed, filtered and recrystalized using ethanol. Reaction involved is shown in Fig.1

Metals pre-concentration method

Batch method

An aliquot of sample solution (100 ML) containing 0.1ìgml^{-1} of any of these metals i.e. Co (II), Cu (II), Ni(II), Zn(II) and Cd(II) was taken in a glass stoppered bottle (250 ml). Before taking these aliquots the pH is adjusted to the optimum value. Then 0.1g of HPEAHBA impregnated activated carbon is added to the bottle and the mixture was shaken for 30min. After filtration, the sorbed metal ion was eluted with 6.0ML of 1M HNO3 in acetone. The concentration of metal ion in the eluate was determined by a pre-standardized FAAS

Column method

Activated carbon (AC) loaded with HPEAHBA (1.0g) was packed in a glass column (1.0x10cm^2) and treated with 1M HNO_3 in acetone and washed with double distilled water until the AC was free from acid. A suitable aliquot of the solution containing Co (II), Cu (II), Ni (II), Zn (II) and Cd (II) was passed through the column after adjusting its pH to an optimum value at a flow rate of 2.5-4.5$mLmin^{-1}$. The column was washed with double distilled water to remove free metal ions. The eluate of the metal ions from the AC was carried out by 1M HNO_3 in acetone. The eluate was collected in 25mL calibrated flask and made up to the mark with double distilled water. Finally, this aliquot was aspirated into the nebulizer of FAAS for determination of trace metals in environmental samples.

Determination of metal ions in water samples

AC-HPEAHBA was used to preconcentrate the Co(II), Cu(II), Ni(II), Zn(II) and Cd(II) ions in water samples collected from the industrial areas (Doddaballapur) and municipal taps (Chickballapur), followed by their determination with FAAS. The estimation of all these metal ions was made with and without (referred as direct determination) standard addition (S.A) by passing 1000mL of water sample (spiked with 50-100 µg of each of the five metal ions in the case of standard addition method) through

Table-1:

Instrumental conditions for the determination of Co, Cu, Ni, Zn and Cd

Conditions	Characteristics	Metal	Wavelength (nm)
Flame: Acetylene	2.0 (L/min)	Cobalt	240.7
Air	1.5 (L/min)	Copper	324.8
Hallow cathode lamp	Photonics L233 Lamp	Nickel	232.0
Lamp current (mA)	12	Zinc	213.9
Slit Width (nm)	0.5	Cadmium	228.8
Burner height (mm)	7	Measurement mode	Background Correction

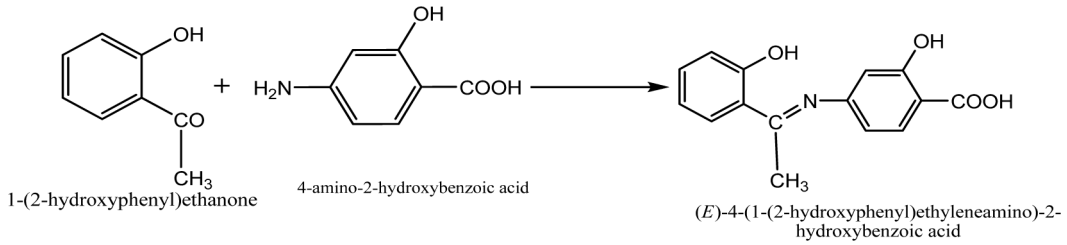

Figure -1

the column packed with 1.0g of matrix after adjusting the pH to an optimum value and determining the metal ion as described in the recommended column procedure. The elution made with 1M HNO_3 in acetone was used. The results given in Table 6 reflect the suitability of the AC for water analysis. The concentration reported in Table 6 as estimated by standard addition method are the values obtained by subtracting the amount of metal added for spiking from the total metal recovered. The closeness of results of direct and SA method indicates the reliability of present method of metal analyses in water samples.

Determination of Co in pharmaceutical samples

Solid phase extraction with AC-HPEAHBA coupled with FAAS method of determination was applied to determine cobalt in pharmaceutical samples. The contents of vitamin B12 as cobalt in four ampules of injection were decomposed in a 50mL round – bottom flask by heating with a 5.0mL mixture containing concentrated nitric and sulfuric acids (10:1) on a hot plate until near to dryness [30]. A drop wise addition of concentrated nitric acid was needed to obtain a colorless residue. The residue was neutralized with a dilute sodium hydroxide solution, and was then diluted to 50mL volume in a standard flask. The cobalt contents were analyzed using 2.0mL of the solutions by the recommended procedure. A standard method using Nitroso-R salt has also been used [31], as a reference method. The results are given by Table .2

Table-2:
Determination of cobalt in B12 vitamin

Sample	Cobalt found [a]/ µgmL^{-1}RSD, % (n=4)	Cobalt found [a] µgmL^{-1} RSD, %(n=4)
1	44.9 ±1.2	45.3±2.4
2	46.8 ±0.9	46.7±1.5

Determination of Zn in a milk sample

A sample of powdered milk (1.0g) was heated in a beaker containing mixture of concentrated sulfuric acid (10mL) and nitric acid (4mL) till a clear solution was obtained. It was allowed to cool and most of the acid was neutralized with 2mL in sodium hydroxide. The pH was adjusted to optimum value and the volume was made up to 500mL in standard flask. The concentration of zinc was estimated by passing the solution through the column packed with HPEAHBA loaded AC. The metal ion was eluted from the column using 2 mol L $^{-1}$ HCL (as per recommended procedure) and determined using FAAS. The average (three determinations) amount of zinc was found to be 38.55µg g^{-1} (R.S.D. ~ 4.28%). The reported of zinc in the milk sample as residue is 38.0 µg g^{-1} as shown in Table 3

Table-3:
Determination of Zinc

Sample	Zinc found (µg g^{-1})	RSD, %(n=3)
1	38.52	4.28
2	38.58	4.29

Results and Discussion

Effect of p^H

P^H is an important parameter, because it significantly affects the metal –AC-HPEAHBA complex formation. The effect of pH on complexation of metal ions with AC-HPEAHBA was studied by adding 100 µg of each of element individually in 150mL double distilled water and determined by complexing with AC-HPEAHBA in the pH range of 2.0-7.0. The result shown in Fig.2. indicate maximum recovery at pH 5.0 ± 0.2 for all the elements. So, pH 5.0 ± 0.2 was selected for further investigations.

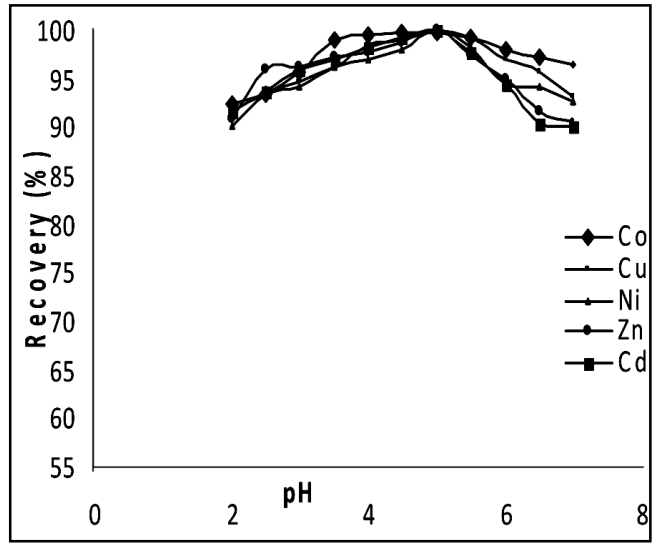

Figure -2

Effect of sample volume

The effect of sample volume on the elution of Co, Cu, Ni, Zn and Cd was studied by taking different volumes of various samples, 100, 200, 300, 400, 500, 600 and 700mL. The extraction was carried out as described in the earlier procedure. In all cases the recovery obtained was higher than 98.5% for all these elements. The results are shown in Fig.3. However, the efficiency of recovery slightly decreases when the sample was chosen for the present study

Effect of flow rate of sample volume

The degree of metal ion sorption on AC-HPEAHBA was studied by varying the flow rate of the metal ion solution (sample solution). The optimum flow rate for loading all these metal ions was 0.5-3.0mL min^{-1}. As flow rate increased beyond 3.0mL min^{-1}, there was a decrease in

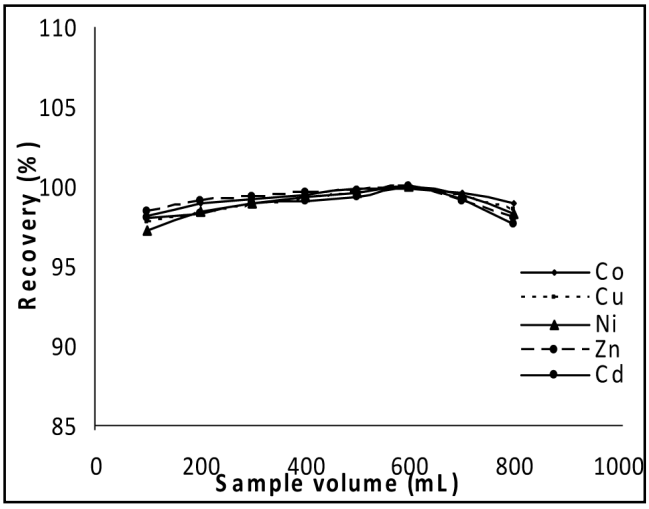

Figure -3

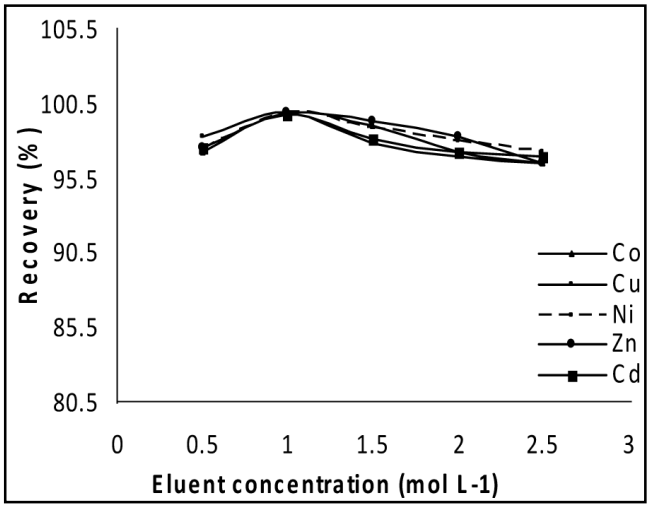

Figure -4

the percentage of sorption of metal ions. The results are shown in Fig.4. Hence, 3.5mL of sample solution was chosen for further investigations.

Total sorption capacity

A volume of 150mL solution containing 100 µg of each metal (pH 5.0 $\pm$ 0.2) was placed in contact with 0.5 g of AC-HPEAHBA at constant stirring (rpm) during 24 h and the sorption capacity of the AC-HPEAHBA was determined by column method and shown in Table.4. The solid matrix was filtered and washed with double distilled water. Then the sorbed metal ions were eluted with 6.0mL of 1M HNO_3 in acetone and estimated by FAAS to determine the sorption capacity of the column. The batch method was also used to determine the sorption capacity and similar result were obtained. It was found to be nearly same (variation <5%) by the two methods.

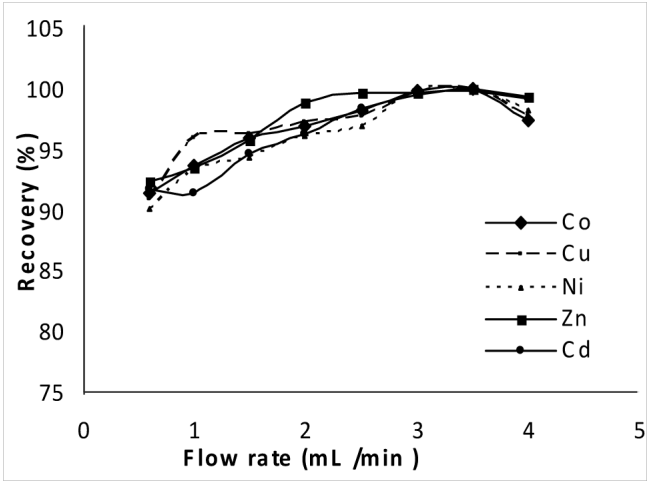

Figure -5

Preconcentration efficiency

The efficiency of the AC-HPEAHBA column for the sorption of metals was studied by using 450mg of AC-HPEAHBA and comparison with 450mg of AC for pre-concentration of metals in a model solution. Starting with 40ìg of each metal in50ml of solution, the quantity of unretained metals in the filtrate was determined by FAAS. The percentage sorption of the metals retained on the sorbents was calculated from the difference between starting amount of each metal (mg)(N_s) and the amount of metal (mg) left in the filtrate (N_f). The AC-HPEAHBA can retain all the metal ions while the untreated AC cannot quantitatively retain Co, Cd, Ni, and Zn. Evidently, the

Table-4:
Experimental Parameters

Experimental parameters	Metal ions				
	Co (II)	Cu (II)	Ni (II)	Zn (II)	Cd (II)
PH range	5.0	5.0	5.0	5.0	5.0
Flow rate (ml min^{-1})	0.5-2.0	1.5-3.0	2.5-3.5	0.5-2.0	1.0-2.0
Sorption capacity (ì mol^{-1}	225	470	255	200	100
Average recovery (%)	99.7	98.8	98.9	99.0	98.2
Standard deviation	0.050	0.038	0.025	0.030	0.038
Relative standard deviation (%)	4.395	3.788	2.285	2.755	4.022

pre-concentration of the metals with the untreated AC is not suitable for Co, Cd, Ni, and Zn. Therefore, AC-HPEAHBA seems to be better sorbents in simultaneous sorption of the studied elements at pH 5.0±0.2.

Preconcentration and recovery of metal ions

Enrichment factor was determined by increases the dilution of metal ion solution increasing metal dilution while keeping the total amount of loaded metal ion fixed at 15ìg for Cd and 20 ìg for Co, Cu, Zn or Ni and applying the recommended column procedure. The Preconcentration factors for Co (II), Cu (II), Ni (II), Zn (II) and Cd (II) are 167, 290,100,290 and 250, respectively, are shown in Table 5

Method of evaluation

The proposed column Preconcentration solid phase extraction method was critically evaluated with regard to reproducibility, accuracy and detection limit.

Reproducibility

To test the reproducibility of proposed column solid phase extraction method, four repetitive analysis cycles of each sample were run. A % R.S.D.in the range 0.6-6.0 was obtained as shown in Table.2 and 6.

Accuracy

The accuracy of proposed column Preconcentration solid phase extraction method was evaluated by comparing the results with those obtained by the reported method (32). The results shown in Tables.2 and 6 reveals good correlation between the two methods indicative of present method is more sensitive than the reported method in the literature (32)

Detection limits

Under optimized conditions the detection limits for the determination of metal ions using column Preconcentration solid phase extraction method was presented in Table.7.

Effect of electrolytes and cations

The effect of electrolytes NaCl, NaF, $NaNo_3$, Na_2So_4, Na_3PO_4, NaI and other foreign species on the sorption of Co(II), Cu(II), Ni(II), Zn(II), and Cd(II) onto HPEAHBA-AC matrix was studied. A species is considered to interfere when it lowers the recovery of metal ions more than 2.5% in comparison to the value observed in its absence. The tolerance limits of various foreign species in the sorption of all the metal ions were studied .These values indicate that sorption on HPEAHBA – AC is not much sensitive to foreign species.

Table-5:

Enrichment factors the determination of trace metals

Metal Ion	Total volume (mL)	Concentration (ìgml^{-1})	Final volume	Recovery (%)	Preconcentration factor
Co (II)	2500	8.0	15	98.2	175
Cu (II)	3000	6.7	10	98.3	300
Ni (II)	1000	20.0	10	98.3	100
Zn (II)	3000	5.0	10	98.6	299
Cd (II)	2500	10.0	10	97.6	246

Table-6:

Determination of metal ions in water samples

Sample collected	method	Metal ion (ìg ml^{-1})				
		Co (R.S.D)	Cu (R.S.D)	Ni (R.S.D)	Zn (R.S.D)	Cd (R.S.D)
River water	Direct S.A.	12.8±1.9	19.9±1.2	6.4±3.2	3.6±3.2	4.0±6.0
		13.0±0.8	20.1± 0.95	6.4± 3.0	3.1± 5.6	4.3± 3.4
Tap water[b]	Direct S.A.	14.6± 0.9	24.3±1.3	12.6±1.4	14.8± 1.6	7.4±2.6
		14.9±1.2	24.4±0.6	13.4±1.3	14.5± 0.2	7.0±1.4

Direct, recommended procedure is directly applied; S.A., standard addition method; R.S.D.(%),for four determinations [a] River water collected Doddaballapur industrial area, [b] Tap water collected from Chickballapur

Table-7:

Detection limit for the determination of Co, Cu, Ni, Zn and Cd

S. No.	Element	Detection limit (μgmL^{-1})
1	Co	1.09
2	Cu	0.75
3	Ni	1.72
4	Zn	1.01
5	Cd	3.82

Applications

To evaluate the applicability of the Preconcentration and solid phase extraction of metal ions, it was applied to the determination of Co (II), Cu (II), Ni (II), Zn (II), and Cd (II) in pharmaceutical, water and milk samples. The analytical data summarized in Tables.2 and 6 suggest that the percentage of the recovery of metal ions ranges from 98.50 to 99.82 % which is more reliable and sensitive than the metal reported in the literature. It is evident from the data in Table.8 that the proposed method is rapid, economical and more sensitive.

Table-8:

Comparison of the present method with the reported methods

Reagent	Instrumentation	Detection Limit (μgml^{-1})	References
Diethyldithiocarbamates	FAAS	4-23	(25)
ChloromethylatedPolystyrene-PAN	FAAS	1-8	(26)
Pyrrolidine dithiocarbamate	FAAS	19-28	(27)
2-Aminoacetyl thiophenol	ICP-AES	10-58	(28)
Present method	FAAS	0.75-3.82	present method

Conclusion

The sorption capacities of the present method are compared with those of other chelating matrices. It shows in some cases higher capacities in comparison to others may be obtained in terms metals with very few exceptions. A simultaneous Preconcentration method for Co(II), Cu(II), Ni(II), Zn(II), and Cd(II) from aqueous solutions on using an activated impregnated with 4-(1-(2-hydroxyphenyl) ethylideneamino)-2-hydroxybenzoic acid column and batch methods were developed. The results obtained shows that the proposed method can be applicable for the determination of trace metal ions in variety of environmental and pharmaceutical samples with low detection limit, high accuracy and precision.

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Rapid Synthesis of New-2-thioxodihydropyrimidine and Its Mannich Bases- Tautomeric Studies and Pharmacological Evaluation

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Abstract

Ethyl 4-(4-methylphenyl)-6-methyl-2-thioxo3,4-dihydropyrimidine-5-carboxylate was synthesized by the conventional and rapid MWI methods using calcium chloride as acid catalyst and characterized through its analytical and spectral (IR,^{1}HNMR and mass) data. Then it was subjected to the Mannich condensation with formaldehyde and one of the six different secondary amines, in the presence of potassium carbonate. The products from such reaction were isolated, purified and characterized as their respective S-Mannich bases involving N^1-H specifically in thione–thiol tautomerism, based on elemental and spectral analyses. Both, solvent-free thermal method and MWI method were successful in yielding the product, the latter being more rapid. The new Mannich bases were evaluated for their analgesic and anti-inflammatory properties by standard methods in animal models and all of them were found to exhibit moderate potency in comparison to aspirin and phenyl butazone.

Key words: Mannich bases, microwave irradiation, thione-thiol tautomerism, analgesic. anti-inflammatory

Introduction

Like dihydropyrimidines, the analogous and isosteric dihydropyrimidines scaffold has been proved to be potential. Though literature reveals a voluminous work on various 4-aryl-dihydropyrimidinones and their derivatives [1-10], a detailed survey of the family reveals an interesting fact that no work has been reported, so far either on the Mannich bases of 4-(4-methylphenyl)3,4-dihydropyrimidin-2(1H)-ones or their thioxo analogues. Monostrol paved the way to work on thioxo pyrimidines and pharmacological importance of a prototype and in addition our previous experience of investigating the tautomeric nature of some heterocyclic systems like benzimidazoles and benzimidzol-2-thiones [11-15], 1,4-dihydropyrimidines and 3,4 dihydropyrimidinones has been quite encouraging to investigate further the heterocyclic Mannich bases in particular associated with potent pharmacological properties. Therefore in continuation of our work on nitrogen heterocycles and DHPMs, in particular and to synthesize some new S-Mannich bases while studying the thiol-thione tautomerism involving either of the two N-H systems, the synthesis and Mannich condensation of a thioxo-DHPM: 5-carboethoxy6-methyl-4-(4methylphenyl)-3,4-dihydropyrimidin-2-thione has been chosen, worked out experimentally and the results are presented herein.

The one-pot Biginelli reaction has been carried out using a three-component mixture: 4-methylbenzaldehyde, ethyl acetoacetate and thiourea under solvent-free conditions with fused calcium chloride as an acid catalyst, by both thermal and MWI methods. The product obtained from either of the methods has been further purified by recrystallisation from aqueous ethanol to get a white crystalline solid m.p.193-194^{o}C.The compound has been characterized as 5-carboethoxy4-(4-methylphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione or ethyl 4-(4-methylphenyl)-6-methyl-2-thioxo-3,4-dihydropyrimidin-5-carboxylate (Scheme-1 ,IV). On the basis of spectral (IR,^{1}HNMR, and mass) data supported by its satisfactory analytical data [16].

The Compound IV has then been subjected to the Mannich condensation with aqueous formaldehyde(37%), potassium carbonate and one of the six different secondary amines(V) in each reaction, again under two different sources of energy viz., thermal and MWI, while monitoring the reaction by TLC. The work up of the reaction mixture

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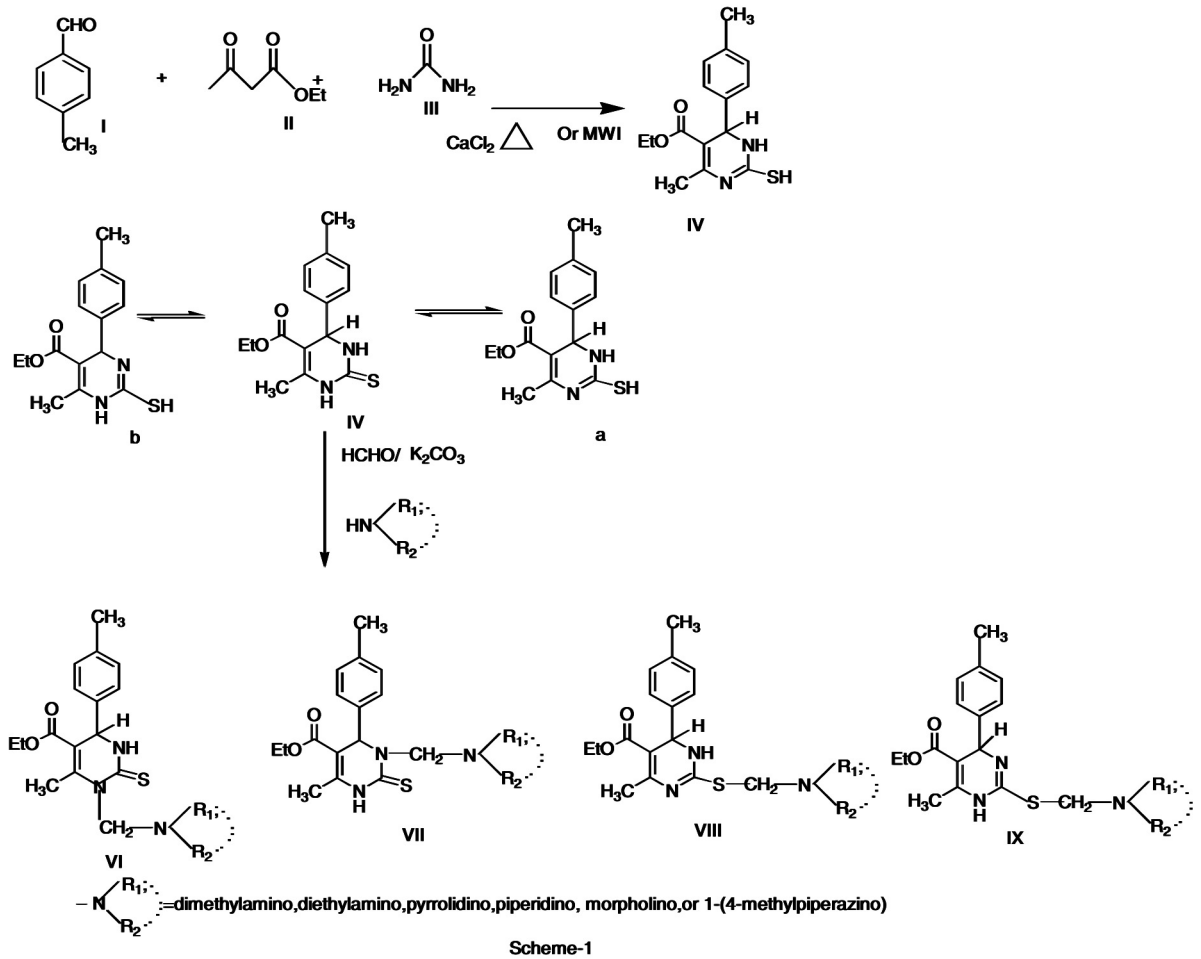
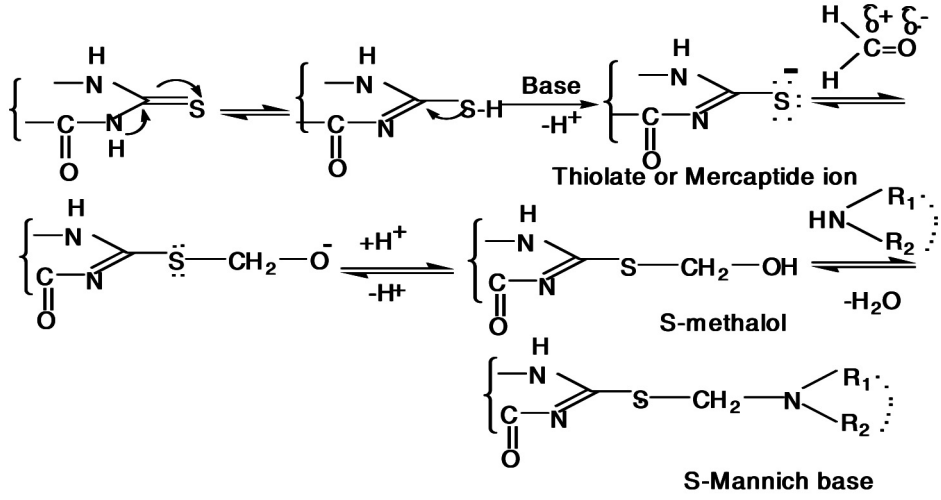


Scheme-1

has resulted in single product in each case. It could be either N^1-(VI), N^3-(VII) or S-Mannich bases of DHPMs(VIII or I)in view of ureido-NH groups and the possible thione-thiol (IVa, IVb) tautomerism of the substate molecule (Scheme-1)

It could be observed from the IR and ^{1}HNMRspectra of the product molecules,compared with that of the substrate, one of the N-H has disappeared indicating its involvement in the Mannich reaction either itself directly or indirectly by shifting itself to sulphur, in the form of thiol (IVa or IVb). In basic medium (in K_2CO_3), it could be visualized that there is a greater possibility for thione-thiol tautomerism to form preferably the thiolate or mercaptide ion. The thiolate anion being a good nucleophile, is then expected to involve in the Mannich condensation to form a corresponding S-Mannich base (VIII or IX) and not N-Mannich base (VI or VII).



Then out of the possible product molecules VIII or IX it can be characterized specifically as that of the structure VIII and not IX on the basis of the ^{1}HNMR spectra of all such products. The^{1}HNMR spectra of the compounds obtained showed a characteristic doublet at ä 5.2 to 5.6 ppm with a coupling constant (J) of 3.2 to 3.6 Hz as could be seen in the substrate (DHPM) spectrum. This is assignable to the C_4-H which is split with the neighbouring N^3-H proton. Further the N-H signal in the downfield (ä 8.85 to 9.30 ppm) characteristic of N^1-H of the dihydropyrimidines, is significantly missing while that of the N^3-H (ä 6.5 to7.8 ppm) being recorded. Thus, it could be concluded that N^1-H being more labile is preferably involved in the thiol (IVa) formation and not the N^3-H. Therefore, the product molecule can be characterized as the respective 5-carboethoxy2-(substituteda mino) methylthio-6-(4-methylphenyl)-4-methy-1,6-dihydropyrimidine (VIII).

Experimental

Purity of the compound was checked on TLC, melting points were determined using Polmon melting point apparatus and are uncorrected. Infrared spectra were recorded (in KBr pellet) on Perkin Elmer FT-IR Infrared Spectrophotometer (Spectrum-1 model) while ^{1}HNMR and ^{13}C-NMR spectra, on Bruker-300MHz. The mass spectra of the compounds were obtained on Schimadzu LC Mass Spectrometer.

Synthesis of 5-carboethoxy4-(4-methylphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione(IV):

Solvent-free Thermal Method: 4-Methylbenzaldehyde (**I**, 12.0g; 0.1mol) was stirred with ethyl acetoacetate (**II**, 13.0g; 0.1mol) for 30 minutes and thiourea (**III**, 11.40g; 0.15mol) along with fused calcium chloride (1.1g; 0.1eq) was introduced. The reaction mixture was heated in an oil-bath under reflux for 1 hr, while stirring the progress of the reaction was monitored by frequent TLC studies, the reaction mixture was cooled to room temperature a solid starts separating, and then crushed ice was added while stirring vigorously. The colourless product was filtered, washed 4-6 times with cold water and vacuum dried. Though it was pure enough, purified further by recrystalisation from aqueous ethanol to obtain a colourless crystalline solid (28.10g; yield: 98.5%); m.p.193-194^{o}C. (Lit[16] 190-192^{o}C; 192-194^{o}C)

IR (KBr): 3325.25, 3174(N-H), 2980.78(C-H), 1673.70 (C=O), 1575.14, 1465.6 (C=C), 1370.83 (C-O), 1175.66, 1119.05, 1029.37 Cm^{-1}.

HNMR($CDCl_3$) (ä,ppm) :1.173 (**t**,3H,J=7.2Hz,CO_2-CH_2-**CH_3**), 2.312(**s**,3H,Ar-CH_3), 4.084(**q**,2H,J=7.2Hz,CO_2-**CH_2**-CH_3), 5.345(**d**,1H,J=3.1Hz,C_4-**H**), 7.096-7.184 (**m**,1H,Ar-H), 5.721 (**s**,1H,N^3-**H**) and 8.063(**s**,1H,N^1-H).

Mass (ESI-MS): m/z: 291.99 (100%) [M^++1], 292.12[M^++2], 233.14, 216.10, 200.13, 187.09, 170.15.

Rapid MWI-Method: An intimate mixture of 4-methylbenzaldehyde (**I**, 1.20g; 0.01 mol) , ethyl acetoacetate (**II**, 1.30g; 0.01mol), thiourea(**III**, 1.14g; 0.015 mol) and freshly fused calcium chloride (0.11g,0.1%) was transferred into a dry beaker (100ml), an inverted funnel with a cotton plug in the stem was placed over the beaker and the reaction was subjected to microwave irradiation for 2-5 min at 210 W with half a minute pulses using LG Little Chef domestic microwave oven. It was cooled, crushed ice(10-15g)was added and stirred. The colourless solid separated was filtered, washed with small portions of cold water and dried .m.p:193-194^{0}C; yield: 2.899g (100%).

Synthesis of Dihydropyrimidin-2 thione Mannich bases (VIII): General Method:

Conventional Solvent-free Method: 5-Carboethoxy4-(4-methylphenyl)-6-methyl-3,4-dihydropyrimidin-2-thione(**IV**;2.90g,0.01mol)was stirred, with aqueous K_2CO_3(1.626g,0.012 mole) then with aqueous formaldehyde (37%),an appropriate secondary amine (**V**; 0.012 mol) was introduced and heated under reflux in an oil-bath while stirring mechanically, for 1hr . The reaction was monitored by TLC cooled, and to the reaction mixture added crushed ice (20gm) while stirring.The product was washedfree from alkalinity with cold water. It was dried and purified by recrystallisation from heptane-chloroform mixture.

Rapid MWI Method: A mixture of the reactants as specified above was taken into a dry beaker and stirred magnetically, for 10-15min.The beaker containing reaction mixture was equipped with an inverted funnel and subjected to MW irradiation for 5-Carboethoxy2-[(dimethylamino) methyl]thio-6-(4-methylphenyl)-4-methyl-1,6di-hydropyrimidine(VIIIa,S-N R^1R^2 = dimethylamino). m.p.154^{0}C, Yield:65% (MWI: 78%)

IR (KBr) in Cm^{-1}:3417 (NH), 1694.6 (C=O), 1564 & 1460 (C=C, arom), 1317,1186 (C-O)

HNMR($CDCl_3$)(ä ppm): 1.235(**t**,3H,J=7.1Hz,CO_2-CH_2-CH_3), 1.837[**m**,6H,$(CH_3)_2$] 2.338 (**s**,6H,Ar-CH_3+C_6-CH_3), 4.130(**q**,2H,CO_2-CH_2-CH_3),5.346(**d**,1H,J=2.9Hz,C_6-H),5.843(**m**,2H,-S-CH_2-N),7.0110(**d**,2H,J=8.1Hz,Ar-Hortho), 7.169(**d**,2H,J=2.7Hz, Ar-Hmeta) and 7.824(**s**,1H,N^1-H).

Mass (ESI-MS): m/z: 390.33(20%)[M^++1], 371.98, 345.16, 342.24, 305.22, 303.01, 289.13.

5-Carboethoxy2-[(dimethylamino)methyl]thio-6-(4-methylphenyl)-4-methyl-1,6-dihydro- pyrimidine (VIIIa, -NR^1R^2= diethylamino) m.p. 161^{0}C, Yield:72% .

IR (KBr) in Cm^{-1}: 3412(NH), 1696.6(C=O,ester), 1574 &1465(C=C,arom), 1327,1192 & 1113, (C-O, stretch).

^{1}HNMR($CDCl_3$)(ä ppm): 1.01(**t**,6H,-N-(CH_2-CH_3), 1.10(**t**,3H,CO_2-CH_2-CH_3), 2.42 (**s**, 3H,Ar-CH_3), 2.67(**q**,4H,-N-(CH_2-CH_3), 4.12(**s**,2H,S-CH_2-N), 4.28(**q**,2H,CO_2-CH_2-

CH_3), 4.56(**d**,1H,C_6-H), 7.18(**d**,2H,J=8.0Hz,Ar-Hortho), 7.29(**d**,2H,J=2.8Hz,Ar-Hmeta), 7.82(**s**,1H,N^1-H).

5-Carboethoxy6-(4-methylphenyl)-4-methyl-2-[(pyrrolodin-1-yl)methyl]thio-1,6-dihydropyrimidine (VIIIc, S-NR^1R^2 =1-pyrrolidine) : m.p.112^{0}C ,Yield-78%.

IR (KBr) in Cm^{-1}: 3190.92(NH), 2979.88(C-H), 1691.62 (C=O,ester), 1651.82, 1566.54 (C=N)1520.8 & 1460.86 (C=C,arom), 1315&1275.38, 1175.35 and 1099.90(C-O).s

^{1}HNMR ($CDCl_3$) (ä, ppm): 1.184(**t**,3H,J=7.0 Hz,CO_2-CH_2-CH_3), 1.239 [**m**,8H, $(CH_2)_4$ pyrrolidinine] 2.318 (**s**,6H,C_4-CH_3),2.351(**s**,3H,Ar-CH_3), 4.115(**q**,2H, J=7.0Hz,CO_2-CH_2-CH_3),5.208(**d**,1H,J=3.6Hz,C_6-H),5.549(**s**,2H,-S-CH_2-N), 7.162 [**d**,2H,J=8.0Hz, Ar-H(ortho)], 7.265[**d**,2H,J= 2.85Hz,Ar-H (meta)].

^{13}CNMR(DMSO-d_6) (ä ppm) : 14.109, 18.027, 18.213, 21.137, 55.816, 60.395, 76.451, 76.494, 103.033, 126.703, 127.041, 129.496, 138.113, 139.578, 14.780, 165.349, 174.95.

Mass (ESI-MS): m/z: 374.90(18%) [M^++1], 347.17(32%), 329.16(100%), 283.19(23%), 215.02(23%), 187.15(12%), 169.13(74%),161.10(10%).

5-Carboethoxy6-(4-methylphenyl)-4-methyl-2-[(piperidin-1-yl)methyl]thio-1,6-dihydropyrimidine (VIIId, S-NR^1R^2 = 1-piperidino). m.p.176^{0}C ,Yield:65% .

IR (KBr) in Cm^{-1}: 3417.07(NH), 2978.18(C-H) 1693.60(C=O), 1658.16, (C=N), 1563.91&1460 (C=C, arom), 1316.63&1186.18and1110.65 (C-O).

^{1}HNMR ($CDCl_3$)(ä ppm): 1.107(**t**,3H,J=7.2Hz,CO_2-CH_2-CH_3), 2.27(**m**,8H,morpholinyl) 2.503(**s**,3H,Ar-CH_3), 4.015(**q**,2H,J=7.2Hz,CO_2-CH_2-CH_3), 5.139 (**d**,1H,J=3.2Hz, C_6-H), 7.080-7.16(**d**,7H,Ar-H).

^{13}CNMR (DMSO-d_6)(ä ppm):14.491, 17.599, 21.121, 60.029, 101.309, 126.750, 129.525,

Mass (ESI-MS): m/z: 388.18(18%) [M^++1], 370.07(100%), 357.91(24%), 340.16(56%), 327.99(06%), 303.01(10%)

5-Carboethoxy6-(4-methylphenyl)-4-methyl-2-(morpholinomethyl)thio-1,6-dihydropyrimidine (VIIIe, S-NR^1R^2 = morpholino) m.p.108^{0}C, Yield:71% .

IR(KBr) in Cm^{-1}: 3417.07(NH), 1693.60(C=O,), 1563.91 & 1460.25(C=C,arom), 1316.63, 1186.18 and 1110.65(C-O,stretch).

^{1}HNMR ($CDCl_3$) (ä ppm): 1.18(**t**,3H,J=7.0Hz,esterCH_3), 2.32(**s**,3H,C_4-CH_3), 2.35 (**s**,3H, Ar-CH_3), 2.448-2.55[**m**,4H, $O(CH_2)_2$], 4.130(**q**,2H,J=7.0Hz,esterCH_3), 5.36(**d**,1H, J=3.2 Hz, C_6-H), 7.12-7.27(**m**,4H,Ar-H), 7.824(**s**,1H,N^1-H).

Mass (ESI-MS): m/z: 390.33(20%) [M^++1], 371.99(38%), 345.16(12%), 342.24(18%), 303.01 (100%), 289.13(40%).

5-Carboethoxy2-[(piperazin-1yl)methyl]thio-6-(4-methylphenyl)-4-methyl-1,6-dihydropyrimidine (VIIIf, -NR^1R^2 =1-piperazino).m.p.181^{0}C ,Yield:73%.

IR (KBr) in Cm^{-1}: 3418 (-NH,strecth), 3365(N-H,str), 3078(C-H), 2967(C-H,alip) 1682(C=O,str), 1605 and 1472 (C=C,arom), 1332,1196 and 1115,str (C-O).

^{1}HNMR ($CDCl_3$+d_6-DMSO) (äppm): 0.96(**s**,1H,-NH;piperozino), 1.04(**t**,3H,ester-CH_3), 2.36(**s**,3H,Arom-CH_3), 2.48(**s**,3H,C_4-CH_3), 2.52-2.68[**m**,8H,-N$(CH_2)_4$], 4.06 (**q**,2H, ester-CH_3), 5.10(**d**,1H,C_6-H), 6.48(**s**,1H,N^1-H), 7.12-7.31(**m**,4H,Ar-H).

Pharmacological screening:

In view of the fact that DHPs are known to possess anti-inflammatory properties [17-20] and some heteryl Mannich bases posses analgesic activity (Physical and Chemical methods). Physical: Haffners Tail Clip method and chemical : acetic acid induced - writing method along with that of anti-inflammatory activity(carrageen-induced rat paw oedema method), the new Mannich bases of thioxopyrimidines were evaluated, experimentally in animal models adopting standard procedures [21-24]. The results were presented in Table-1 along with that of aspirin and phenyl butazone, employed as standards, for comparison.

Results and discussion:

The solvent-free Biginelli reaction proceeded well in presence of anhydrous calcium chloride as an acidic catalyst, under both conventional and MW irradiation conditions yielding the 2-thioxodihydropyrimidine in good quantity .Interestingly the yields (98.5% and 100% respectively) have not differed much by varying the experimental conditions except recording a considerably less time in MWI method when compared with thermal heating. It has been characterized satisfactorily on the basis of its analytical and spectral data. The Mannich condensation of the pyrimidin-2-thione has also proceeded very satisfactorily with aqueous formaldehyde (37%) itself in presence of potassium carbonate. The products from all such reactions have been characterized as their corresponding S-Mannich base with the help of analytical and spectral data.

The pharmacological screening of the new S-Mannich bases for their possible analgesic and anti-inflammatory properties, by standard methods, in experimental animals have revealed the following:

All the S-Mannich bases of 4-p-tolyl were found to exhibit a moderate analgesic and anti-inflammatory properties when compared with that of standard aspirin and phenyl butazone. The results on analgesic activity clearly indicate that the present compounds exhibit almost same degree of protection irrespective of the cause of analgesia (physical and chemical).The S-Mannich bases

Table-1:

Analgesic & antiinflammtory data of S-Mannich basesof ethyl 4(4-methylphenyl)-6-methyl-2-thioxo-3, 4-dihydropyrimidin-5-carboxylate

Compound No	S-Substituent S-NR_1R_2	Analgesic Activity* (%Protection)		Anti-inflammatory activity* (% odema inhibited)
		Tail-clip	Writting	
VIIIa	Dimethylamino	35	38	42
VIIIb	Diethylamino	32	30	34
VIIIc	1-pyrrolidino	33	32	36
VIIId	1-piperidino	40	43	38
VIIIe	4-morpholino	42	40	42
VIIIf	1-piperazino	35	36	35
Aspirin	Standard	68	60	62
phenylbutazone	Standard	-	-	58

*Note: Both the assays were carried with a test dose of (100mg/kg (b w).

with piperidino and morpholino groups were relatively more potent against the rest of the test compounds. As far as the anti-inflammatory activity is concerned the S-Mannich bases with dimethylamino and morpholino groups were found to exhibit a moderate potency in comparision to phenyl butazone; the next in order being the piperidino compound.

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Antioxidant and Anthelmentic Activity of Vanillin Derivatives

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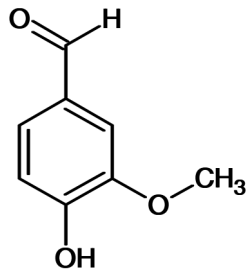
Abstract

The present study was designed to synthesize various vanillin derivatives. The synthesized compounds were subjected for and antioxidant activity anthelmentic activity. ABTS , DPPH, nitric oxide radical scavenging methods were used to evaluate antioxidant activity and anthelmentic activity was performed using Indian earth worms (Pheretima postuma). Ascorbic acid and albendazole was used as standard drug for antioxidant activity and anthelmentic activity. Among the vanillin derivatives compounds IX and III have shown good antioxidant activity in all the three methods employed. The results of anthelmentic activity suggests that all the synthesized compounds posses good paralytic effect but has taken long time to cause death when compared with the standard drug.

Keywords: Vanillin derivatives, Antioxidant, Anthelmentic activity,

Introduction

Free radicals are found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species (ROS) are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation etc [1,2]. The human body although continuously produces free radicals, it possess several defense systems, which are constituted of enzymes and radical scavengers. These are called "first – line antioxidant defense system", but are not completely efficient because almost all components of living bodies, tissues, cells and genes undergo free radical destructions [3]. Thus antioxidants are provided as supplements to suppress oxidative damage. Many synthetic antioxidants are available in the market but due to its toxic and carcinogenic effects they are substituted with natural antioxidants [4].



Vanillin $C_8H_8O_3$ (4-hydroxy-3-methoxybenzaldehyde), is a white crystalline solid, which melts at 81°C. It is the primary component of the extract of the vanilla bean. Vanillin is used as flavoring agent in foods, beverages and pharmaceuticals. Researchers have proved that vanilla posses many pharmacological activities like antioxidant activity, anticancer activity, antimicrobial activity, antidepressant activity, aphrodiasic activity. Recently many researchers claim that vanillin can be used in treatment of sickle cell anemia. It has beneficial effects in patients suffering from alzhemiers and parkinsons disease. Researchers proved that oxidative stress is the major cause for various ailments and since vanillin has proven to have good antioxidant activity. The present study was designed to synthesize vanillin derivatives and to evaluate its antioxidant and anthelmentic activity.

Materials and Methods

Chemical and reagents

Vanillin (S.D.Fine Chem Pvt.Ltd,Boisar), Hydrazine Sulphate (IDPL, India), Phenylhydrazine, Sodium acetate.HCl, Iodine, 2,4-dinitro phenylhydrazine (S.D.FineChem.Pvt.Ltd,India), Hydroxylamine.HCl, Semicarbazone (Sisco research lab, India) *o*-Phenylene diamine (Oxfordlab,India).ABTS [2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid)] diammonium salt (Sigma Aldrich Co, St Louis, USA) and p-nitroso dimethyl aniline (p-NDA) (Acros Organics, USA). Ascorbic acid, Nitro blue tetrazolium (NBT) SD Fine Chemicals Ltd., Mumbai, India. All other chemicals used were of analytical grade.

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Procedure for synthesis of compound III (4[(E)-Hydrazinylidenemethyl]-2-Methoxyphenol) from Vanillin[5].

4-[(E)-hydrazinylidenemethyl]-2-methoxyphenol (Compound III) was synthesized by dissolving 0.5 gm of hydrazine sulphate and 0.8 gm of sodium acetate in 5 ml of water. To the above mixture a solution of 2-5gm of vanillin in ethanol free from aldehydes and ketones was added. The solution was shaken until a clear solution is formed (add little more ethanol if necessary), warm on a water bath for 10-15 minutes and cool until the crystals appear, filter off the crystals and recrystallize from dilute ethanol. m.p. 178°C.

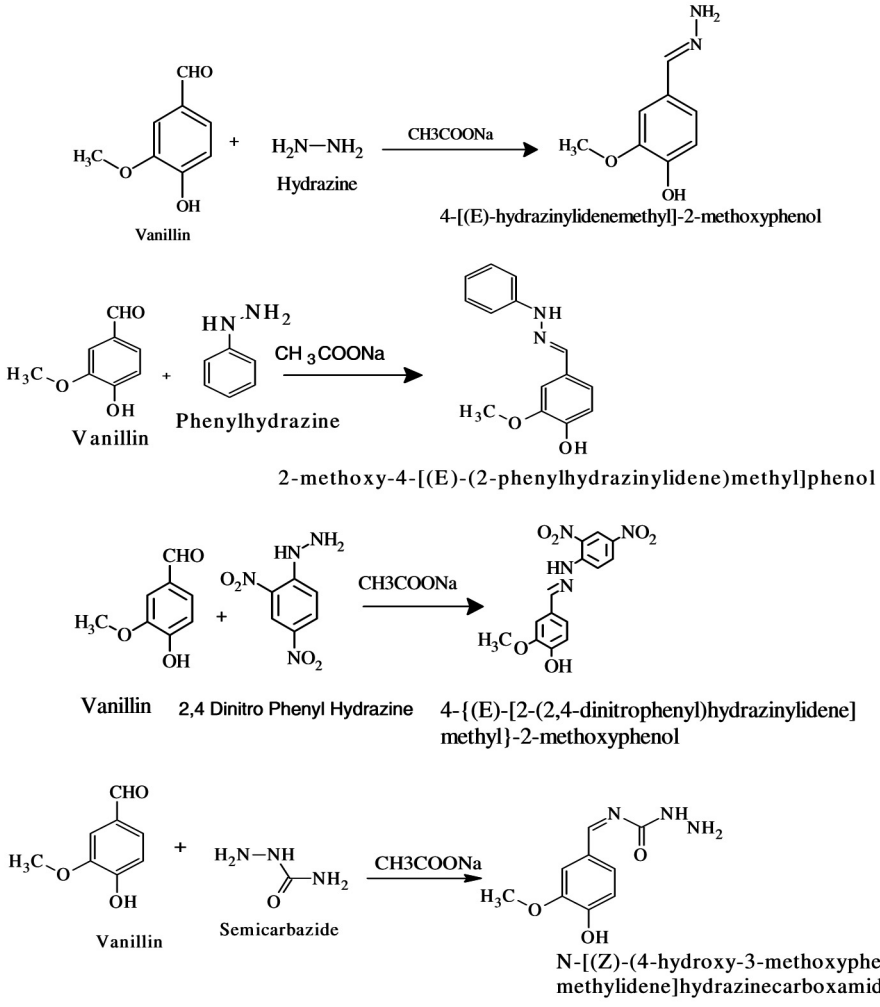
Spectral datas: FT-IR phenolic OH(Str) at 3477.13cm^{-1}, CH(Str) at2928.55cm^{-1}, C=N at 1597.27cm^{-1}, NH (deformation) at 1507.80 cm^{-1}, Aromatic CH deformation at 809.74 &750.86cm^{-1}. ^{1}HNMR signals (δ ppm) at 8.1s (2H- NH_2), 7.0m (1H-Ar-H), 6.7 q (1H-Ar- H), 5.0s (1H-OH), 3.73s (3H-CH_3). Molecular ion (M^+) m/z at 166.

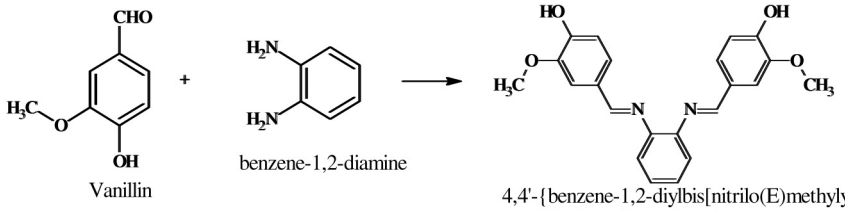
Procedure for synthesis of compound IV (2-Methoxy-4-[(E)-(2-Phenylhydraziny lidene) Methyl]Phenol) from Vanillin[5].

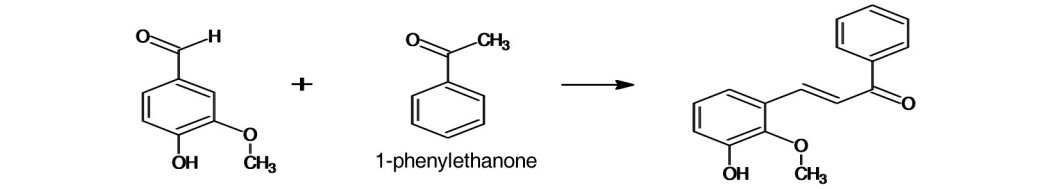
2-methoxy-4-[(E)-(2-phenylhydrazinylidene) methyl] phenol was prepared by dissolving 0.5 gm of colourless phenylhydrazine hydrochloride and 0.8 gm of sodium acetate in 5 ml of water. To the above mixture a solution of 2-5gm of vanillin in ethanol free from aldehydes and ketones was added. The solution was shaken until a clear solution is formed (add little more ethanol if necessary), warm on a water bath for 10-15 minutes and cool until the crystals appear, filter off the crystals and recrystallize from dilute ethanol. m.p.86°C.

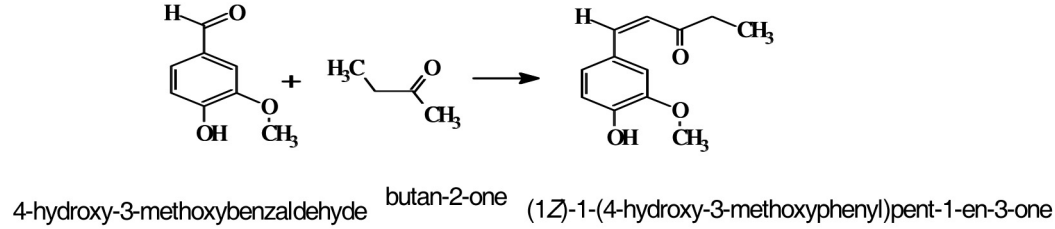
Spectral datas: FT-IR O-H(Str) at3493.59 cm^{-1}, C-H(Str) at 3312.98 cm^{-1}, two aromatic rings at 3043.56 cm^{-1}& 2942.09 cm^{-1}, C=N at 1599.54 cm^{-1}, CH bending at 1355.42 cm^{-1}, C-O (Str) 1159.94, NH(Bending) at 1507.34, Aromatic at 614.88 cm^{-1}. ^{1}HNMR (δ ppm) at 8.1s (1H-Ar-CH) 6.46- 7.01m (8H-Ar-H) 5.0s (1H- Ar- OH), 4.0s (1H-

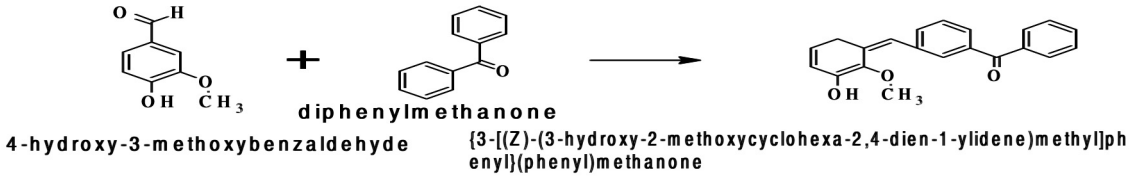
Fig.1 : Scheme for synthesis of vanillin derivatives

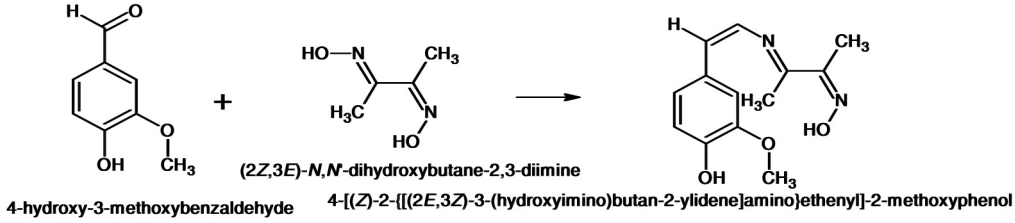












NH), 3.73s (3H-OCH_3). Molecular ion (M^+) peak at m/z at 242.

Procedure for synthesis of compoundV(4-{(E)[2-(2,4-Dinitrophenyl) Hydrazinyl idene]Methyl}-2-Methoxy Phenol) from Vanillin[5].

4-{(E)-[2-(2,4-dinitrophenyl)hydrazinylidene]methyl}-2methoxyphenol was prepared by dissolving 0.5 gm of 2,4 dinitrophenyhydrazine hydrochloride and 0.8 gm of sodium acetate in 5 ml of water. To the above mixture a solution of 2-5gm of aldehyde (vanillin) in ethanol free from aldehydes and ketones was added. The solution was shaken until a clear solution is formed (add little more ethanol if necessary), warm on a water bath for 10-15 minutes and cool until the crystals appear, filter off the crystals and recrystallise from dilute ethanol. m.p. 182°C.

Spectral datas: FT-IR OH(Bending) at 3459.08cm^{-1}, CH (Bending) at 3320.61cm^{-1}, Aromatic ring at 3095.15cm^{-1},C=N at 1582.60cm^{-1}, NH(Bending) at 1504.84cm^{-1}, C=C aromatic (Str) at 1414.12 cm^{-1}, NO_2 (Str) at 1326.37 cm^{-1}.^{1}HNMR signals at (d ppm) at 8.87q (1H-Ar-H), 8.33q (1H-CH), 8.1s (2H-Ar-H), 7.0q (1H-Ar-H), 6.98q (1H-Ar-H), 6.7q (1H-Ar-H), 5.0s (1H- Ar- OH), 4.0s (1H-NH), 3.73s (3H-CH_3). Molecular ion (M^+) peak at m/z at 332.

Procedure for synthesis of compoundVI N-[(Z)-(4-Hydroxy-3-Methylphenyl) Methylidene] Hydrazinecarboxamide from Vanillin[5].

N-[(Z)-(4 - hydroxy - 3 - methoxy phenyl) methylidene] hydrazine carboxamide was prepared by dissolving 1.0 gm of semicarbazine hydrochloride and 10.5gm of crystalline sodium acetate in 8-10 ml of water. To the above mixture

a solution of 2-5gm of vanillin in ethanol free from aldehydes and ketones was added. The solution was shaken until a clear solution is formed (add little more ethanol if necessary), warm on a water bath for 10-15 minutes and cool until the crystals appear, filter off the crystals and recrystallise from dilute ethanol. m.p. 224°C.

Spectral Datas: FT-IR Spectrum of Compound I showed characteristic absorbance peak of NH(S) at 3515.90cm^{-1},OH(S) at 3463.20 cm^{-1}, CH aromatic (S) at 3294.84 cm^{-1}, C=O(S) at 1646.77 cm^{-1} ,C=N(S) 1605.02 cm^{-1},C=C aromatic(S) at 1441.23 cm^{-1} , CH(B) at 1350.76 cm^{-1} .

Molecular ion (M^+) peak at m/z at 209. ^{1}HNMR spectrum showed characteristic signals (d ppm) at 8.1s (1H-CH), 8.0s (1H-NH), 7.0q (2H-Ar-CH), 6.7q (1H-Ar-CH), 5.0s (1H-Ar-OH), 3.73s (3H-OCH_3), 2.0s (2H-NH_2).

Procedure for synthesis of compound VII 4,4'-{benzene-1,2-diylbis[nitrilo(E) methylylidene]} bis(2-methoxyphenol from Vanillin[6].

4,4'-{benzene-1,2-diylbis[nitrilo(E) methylylidene]} bis(2-methoxyphenol) was prepared by the reaction of Vanillin (0.010mol) and *o*-phenylene diamine (0.0050mol) in ethanol was refluxed for 3hrs. The precipitated product was filtered and recrystallised from the ethanol and dried in vacuum over $CaCl_2$. m.p. 182°C.

Spectral datas: FT-IR Spectrum OH(Str) at 3402.91 cm^{-1}, CH aromatic (Str) at 3000.58 cm^{-1}, CH alkane (Str) at 2936.36 cm^{-1}, C=N (Str) at 1599.93 cm^{-1}, NH(Bending) at 1523.63 cm^{-1}, C=C aromatic (Str) at 1456.90 cm^{-1}, C-O (Str) at 1225.80 cm^{-1} .^{1}HNMR signals (δ ppm) at 8.39s (2H-CH), 7.3q (4H-Ar-CH), 7.01q (2H-Ar-CH), 6.96q (2H-Ar-CH), 6.65q (2H-Ar-CH), 5.0s (2H-Ar-OH), 3.73s (6H-OCH_3). Molecular ion (M^+) peak at m/z at 376.

Procedure for synthesis of compound VIII (2E)-3-(3-Hydroxy-2-Methylphenyl)-1-Phenylprop-2-en-1-One from Vanillin[7].

2gm of NaOH in 18ml of water and 9ml of rectified spirit was taken in a 500ml of bolt head flask provide with a mechanical stirrer. The flask was immersed in a bath of a crushed ice, then 4.8 ml of freshly distilled acetophenone was added and the stirring was started, During stirring add 4.2gm of pure vanillin and the temperature of mixture was maintained at about 25^{0}C and vigorous stirring was continued until mixture becomes so thick that stirring is no longer effective. The stirrer was removed and left overnight in an ice chest. The product was filtered with suction and washed with cold water until the washings are neutral to litmus, and then with 20ml of ice cold rectified spirit. m.p. 97°C

Spectral datas: FT-IR Spectrum of N-H (Str) at 3256.27cm^{-1},C=0 bending at 1656.94cm^1. Aromatic C-H bending at 820.03cm^{-1}, Nitro group at 1317.10 cm^{-1}. HNMR signals (δ ppm) at 8.17t (1H-CH), 7.81m (2H-Ar-CH), 7.45-7.54m (3H-Ar-CH), 7.39t (1H-CH), 6.75q (1H-Ar-CH), 6.60q (1H-Ar-CH), 6.50q (1H-Ar-CH), 5.0s (1H-Ar-OH), 3.73s (3H-OCH_3). molecular ion (M^+) peak at m/z at 254.

Procedure for synthesis of compound IX (1Z)-1-(4-Hydroxy-3-Methoxyphenyl) Pent-1-en-3-one from Vanillin[7]

2gm of NaOH in 18ml of water and 9ml of rectified spirit was taken in a 500ml of bolt head flask provide with a mechanical stirrer. The flask was immersed in a bath of a crushed ice, then 4.8 ml of freshly distilled ethylmethyl-ketone was added and the stirring was started, During stirring add 4.2gm of pure vanillin and the temperature of mixture was maintained at about 25^{0}C and vigorous stirring was continued until mixture becomes so thick that stirring is no longer effective. The stirrer was removed and left overnight in an ice chest. The product was filtered with suction and washed with cold water until the washings are neutral to litmus, and then with 20ml of ice cold rectified spirit. m.p. 182°C

Spectral datas: FT-IR N-H bending at 3259.97cm^{-1}, C=0 stretching at 1656.98cm^{-1}, Aromatic C-H bending at 819.48cm^{-1}, Nitro group at 1318.82 cm^{-1}. C=C stretching at1581.23cm^{-1}. ^{1}HNMR signals (δ ppm) at 7.37t (1H-=CH), 6.69q (1H-Ar-CH), 6.64q (1H-Ar-CH), 6.57q (1H-Ar-CH), 6.24t (1H-=CH), 5.0s (IH-Ar-OH), 3.73s (3H-OCH_3), 2.98t (2H-CH_2), 1.11t (3H-CH_3). Molecular ion (M^+) peak at m/z at 206.

Procedure for synthesis of compound X {3-[(Z)-(3-Hydroxy-2-Methoxycyclohexa-2,4-Dien-1-Ylidene) Methyl Phenyl}(Phenyl) Methanone from Vanillin[7]

2gm of NaOH in 18ml of water and 9ml of rectified spirit was taken in a 500ml of bolt head flask provide with a mechanical stirrer. The flask was immersed in a bath of a crushed ice, then 4.8gms of pure benzophenone was added and the stirring was started, During stirring add 4.2gm of pure vanillin and the temperature of mixture was maintained at about 25^{0}C and vigorous stirring was continued until mixture becomes so thick that stirring is no longer effective. The stirrer was removed and left overnight in an ice chest. The product was filtered with suction and washed with cold water until the washings are neutral to litmus, and then with 20ml of ice cold rectified spirit. The crude chalcone after drying in the air weigh 2.7gms and melts at 95^{0}c. Recrystallised using rectified spirit at to 50°C. m.p. 130°C

Spectral datas: FT-IR peak of N-H stretching at 3249.78cm^{-1}, C=0 bending at 1656.58cm^{-1}, Aromatic C-H bending at 819.94cm^{-1},C=C at 1580.97cm^{-1}. Aromatic rings at 1460.55cm^{-}.^{1}.^{1}HNMR spectrum showed characteristic signals (δ ppm) at 13.9s (1H-Ar-OH), 7.74-7.36m 9H-Ar-CH), 6.28q (1H-Ar-CH), 6.16t (1H-=CH), 5.66t (1H-=CH), 3.50s (3H-OCH_3), 2.63d (2H- CH_2). Molecular ion (M^+) peak at m/z at 318.

Procedure for synthesis of compound XI 4-[(Z)-2-{[(2e,3z)-

3-(Hydroxyimino) Butan-2-Ylidene]Amino}Ethenyl]-2-Methoxyphenol from Vanillin[7]

2gm of NaOH in 18ml of water and 9ml of rectified spirit was taken in a 500ml of bolt head flask provide with a mechanical stirrer. The flask was immersed in a bath of a crushed ice, then 4.8 ml of freshly distilled dimethyl ketone was added and the stirring was started, During stirring add 4.2gm of pure vanillin and the temperature of mixture was maintained at about 25^{0}C and vigorous stirring was continued until mixture becomes so thick that stirring is no longer effective. The stirrer was removed and left overnight in an ice chest. The product was filtered with suction and washed with cold water until the washings are neutral to litmus, and then with 20ml of ice cold rectified spirit. Recrystallized using rectified spirit. m.p. 100^{o}C

Spectral datas: FT-IR peak of N-H bending at 3209.26cm^{-1}, C=0 bending at 1581.03cm^{-1}, Aromatic C-H bending at 755.59cm^{-1},C-H bending acyclic at 1361.65cm^{-1}.^{1}HNMR (δ ppm) at 6.69q (1H-Ar-CH), 6.64q (1H-Ar-CH), 6.6t (1H-CH), 6.57q (1H-Ar-CH), 5.2d (1H-=CH), 5.0s (1H-Ar-OH), 3.73s (3H-OCH$_3$), 2.0s (1H-OH), 1.90s (3H-CH$_3$). molecular ion (M$^+$) peak at m/z at 248.

In – vitro Antioxidant studies

A. Scavenging of ABTS radical cation

To 0.2 ml of various concentrations of the test compounds, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution was added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm. The assay was performed in triplicates.[8,9].

B. DPPH Assay procedure for the test compounds:

0.2 ml of DPPH solution was added to 2.8 ml of the test compounds in a test tube wrapped with aluminium foil and its absorbance was read out at 517 nm using UV-visible double beam spectrophotometer. The assay was performed in triplicates.

C. Nitric Oxide Assay procedure for the test compounds:

The reaction mixture (6 ml) containing sodium nitroprusside (10 millimole, 4ml), phosphate buffer saline (PBS, $_p$H 7.4, 1 ml) was added to 2.8 ml of the test compounds and incubated at 25ºC for 15 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 540 nm using UV-visible double beam spectrophotometer [10]. The assay was performed in triplicates.

Ascorbic acid was used as standard in all the three methods and their IC_{50} values were calculated. The data's of the above performed antioxidant activities are represented in Table -1.

***Anthelmintic activity** [11,12]*:

The synthesized compounds were screened for Anthelmintic activity by using earth worms. Six Indian adult earth worms (Pheretima postuma) of nearly equal size 5-8cm in length and 0.2-0.3cm in width were placed in standard drug solution and test compound solutions at room temperature. Normal saline was used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and adjusted the volume up to 15ml with normal saline solution to get the concentration of 0.1%w/v, 0.2%w/v and 0.5%w/v. Albendazole was used as standard drug. The compounds were evaluated for the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time.

To ascertain the death of motionless worms, they were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time and paralysis time of the earth worms for different test compounds and standard drug were tabulated in Table-2.

Results and Discussion

The above synthesized compounds were subjected for invitro antioxidant activity using ABTS, DPPH, Nitric oxide radical scavenging methods. Ascorbic acid was used as standard in all the three methods. The results are expressed in terms of IC_{50} value and represented in Table-1. The result shows that compound I, VI, VII, and IX showed good antioxidant activity when compared with the standard.

Table - 1:

Antioxidant activity of compounds (I-IX)

Compound	IC_{50} Value (µM)		
	ABTS	DPPH	Nitric Oxide
Standard	4.83	5.87	4.02
I	6.98	7.78	7.01
II	10.04	11.00	9.83
III	11.83	9.45	10.78
IV	9.73	10.45	9.68
V	12.89	10.22	11.93
VI	7.83	7.94	6.52
VII	6.95	7.87	5.96
VIII	8.73	7.45	7.04
IX	6.39	7.79	6.62

Anthelmentic activity

All the compounds were screened for Anthelmintic activity by using Indian adult earth worms (*Pheretima postuma*). The compounds were evaluated for the time taken for complete paralysis and death of earthworms by taking albendazole as the standard drug with 0.1, 0.2, and 0.5 % concentrations. The compounds were evaluated and results are presented in Table-2.

Table – 2:

Anthelmintic activity of compounds (I–IX)

Compound	Concentration %w/v	Time in Minutes Mean±SD	
		For paralysis	For Death
Control	0.9%	-	-
Standard	0.1%	49±0.56	68±0.21
	0.2%	44±0.15	62±0.31
	0.5%	38±0.21	53±0.24
I	0.1%	57±0.34	162±0.18
	0.2%	55±0.51	143±0.26
	0.5%	52±0.28	135±0.27
II	0.1%	61±0.31	170±0.36
	0.2%	58±0.25	157±0.15
	0.5%	55±0.36	145±0.34
III	0.1%	63±0.42	178±0.28
	0.2%	59±0.25	162±0.31
	0.5%	57±0.51	149±0.27
IV	0.1%	65±0.24	182±0.54
	0.2%	62±0.32	167±0.51
	0.5%	60±0.29	151±0.34
V	0.1%	67±0.14	168±0.26
	0.2%	63±0.51	149±0.19
	0.5%	59±0.26	140±0.34
VI	0.1%	53±0.34	165±0.28
	0.2%	51±0.31	152±0.35
	0.5%	48±0.24	143±0.34
VII	0.1%	42±0.52	142±0.21
	0.2%	39±0.26	137±0.51
	0.5%	30±0.41	125±0.18
VIII	0.1%	52±0.52	167±0.34
	0.2%	50±0.32	155±0.51
	0.5%	47±0.42	149±0.42
IX	0.1%	61±0.31	170±0.36
	0.2%	58±0.25	157±0.15
	0.5%	55±0.36	145±0.34

Standard: Albendazole

The results of Vanillin derivatives showed that all the compounds have good anthelmentic activity but they have taken more time to cause death when compared with the standard drug (Albendazole). Among the vanillin derivatives, compound X has showed excellent paralytic effect on Indian earth worms but was not as effective as standard drug in causing death.

Thus the results conclude that, Mostly all the molecules showed good activity. Among them Compound III, Compound IX and Compound XIII showed good radical ion scavenging activity in all the three methods. Since antioxidant compounds are likely to posses'anticancer activity. Thus in future these compounds should be subjected for anticancer screening to identify their anticancer activity. The anthelmentic activity showed that all the derivatives have good paralytic effect but was not so effective in causing death, thus the structure has to be optimized in order to derive more potent and safer drug.

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Synthesis, Characterization and Pharmacological Screening of 3-[5-Substituted-1, 3, 4-Oxadiazole-2-yl]-2-Methyl Quinazolin-4(3H)-Ones

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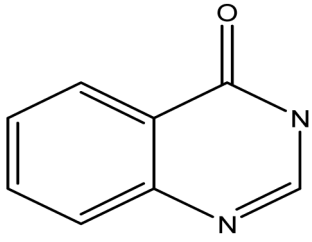
ABSTRACT

A series of 2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino) acetohydrazideVI(a-e)were synthesized and evaluated for their anti-inflammatory by the carrageenan induced paw oedema method and dextran induced oedema and analgesic activity by using hot plate latency and acetic acid induced writhing test the compounds with chloro phenyl group and nitro phenyl group showed excellent anti–inflammatory and analgesic activity when compared with others.

Key words: Quinazolin-4(3H)-ones, anti –inflammatory and analgesic activity.

Introduction

Qunazoline 4-one are an important class of heterocyclic compounds being studied by many researchers and reported to possess a wide spectrum of biological properties such as antitumor agents [1] anticonvulsant, CNS depressant and sedative-hypnotic activity [2] , antibacterial and antifungal activity [3], anti-inflammatory activity [4], analgesic, anti inflammatory and anti bacterial agents [5]. anti viral, anti bacterial and anti hypertensive agents [6, 7], more over Qunazoline 4-one nucleus is a pharmacophore of oxadiazole that occupy a very important place in the field of analgesics and anti inflammatory activity of Qunazoline 4-one nucleus is due to the presence of paracholoro phenyl linkage in its structure in view of these observations a series of scheme with an aim to obtain potential analgesics and anti inflammatory agents were synthesized.



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Materials And Methods

Open capillary method was adopted for determining melting points for the compounds Synthesized using the below mentioned procedures. The purity if the compounds were Determined using Thin layer Chromatography. Thin layer Chromatography was performed using silica gel G (thin layer chromatography grade) and the spots was observed using employing iodine chamber destructive method.

Chemicals and Reagents

Anthranilic acid (manufacturer), pyridine, acetyl chloride5%of sodium bicarbonate, hydrazine hydrate in ethanol *aceto hydrazide* benzoic acid in $POCl_3$ $NaHCO_3$ o-chloro benzoic acid o-nitro benzoic acid p-chloro benzoic acidethanol,

SYNTHESIS

1. *Preparation of 2-methyl 3amino quinqzolin-4(3H) one* IV

Step: 1

To a solution of anthranilic acid (0.1mol) is taken in a beaker and pyridine, acetyl chloride (0.2mol) was added. The reaction mixture is stirred continuously further followed

by 5%of sodium bicarbonate. The solid obtained is recrystalized from ethanol and dried.

Step: 2

A mixture of *2-methyl-4H-benzo[d][1,3]oxazin-4-one* (0.01mole) compound was taken in round bottom flask and treated with hydrazine hydrate in ethanol was refluxed for 3hrs and the resulting solution was poured in to the crushed ice. A white precipitated was obtained and recrystalized with ethanol and dried.

2. ***Preparation of ethyl 2-(4-0xo-2-methyl quinazolin-3(4H)-ylamino) acetate*** V

A mixture of *2-methyl 3amino quinqzolin-4(3H)-one* compound (0.01mole) was taken in round bottom flask and treated with chloro ethyl acetate (0.01mole), DMA, acetone, potassium acetate, and refluxed for 6hrs and the resulting solution was poured in to crushed ice, precipitated was obtain, filtered and recrystalized with ethanol for two times and dried.

3. ***Preparation of 2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino) acetohydrazide*** **VI**

A mixture of *ethyl 2-(4-0xo-2-methyl quinazolin-3(4H)-yl-amino) acetate* compound (0.01mole) was taken in round bottom flask and treated with hydrazine hydrate (0.01mole), in ethanol refluxed for 3hrs and the resulting solution was poured in to crushed ice, precipitated was obtain, filtered and recrystalized with ethanol for two times and dried.

4. ***Preparation of 3-((5-phenyl-1,3,4-oxadiazol-2-yl) methylamino)-2-methylquinaz- olin-4(3H)-one*** **VII(a)**

A mixture of *2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino) aceto hydrazide* compound (0.01mole) was taken in round bottom flask and treated with benzoic acid in $POCl_3$ was refluxed for 5hrs and the contents were cooled and poured in to crushed ice. Then it was neutralized with $NaHCO_3$ solution and resulting solid was filtered and recrystalized with ethanol and dried.

5. ***Preparation of 3-((5-phenylhydroxyl-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin- 4(3H)-one*** **VII(b)**

A mixture of *2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino)acetohydrazide* compound (0.01mole) was treated with salicylic acid in $POCl_3$ was refluxed for 5hrs and the contents were cooled and poured in to crushed ice. Then it was neutralized with $NaHCO_3$ solution and resulting solid was filtered and recrystalized with ethanol and dried.

6. ***Preparation of 3-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3H)-one*** **VII(c)**

A mixture of *2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino) aceto hydrazide* compound (0.01mole) treated with o-chloro benzoic acid in $POCl_3$ was refluxed for 5hrs and the contents were cooled and poured in to crushed ice. Then it was neutralized with $NaHCO_3$ solution and resulting solid was filtered and recrystalized with ethanol and dried.

7. ***Preparation of 3-((5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3H) -one*** **VII(d)**

A mixture of *2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino) aceto hydrazide* compound (0.01mole) treated with o-nitro benzoic acid in $POCl_3$ was refluxed for 5hrs and the contents were cooled and poured in to crushed ice.then it was neutralized with $NaHCO_3$ solution and resulting solid was filtered and recrystalized with ethanol and dried.

8. ***Preparation of 3-((5-(4-chloropheny)l-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin- 4(3H)-one*** **VII(e)**

A mixture of *2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino) aceto hydrazide* compound (0.01mole) treated with p-chloro benzoic acid in $POCl_3$ was refluxed for 5hrs and the contents were cooled and poured in to crushed ice.then it was neutralized with $NaHCO_3$ solution and resulting solid was filtered and recrystalized with ethanol and dried. (Scheme)

Acute Toxicity Studies:

The acute oral toxic study was done according to the OECD guidelines on Acute Oral Toxicity under a computer guided Statistical Programme- AOT423 stat programme, The animals were monitored for the be havioural changes, weight variation, toxicity and death rate.

Animals:

Albi no mice weighing 200–250 g, supplied by M/s. B.N. Ghosh & co., Calcutta, India, were placed in cages with wire-net floors in a controlled room temperature 29°C, relative humidity 60–70 % and provided with food and water *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. All studies were carried out by using six rats in each group.

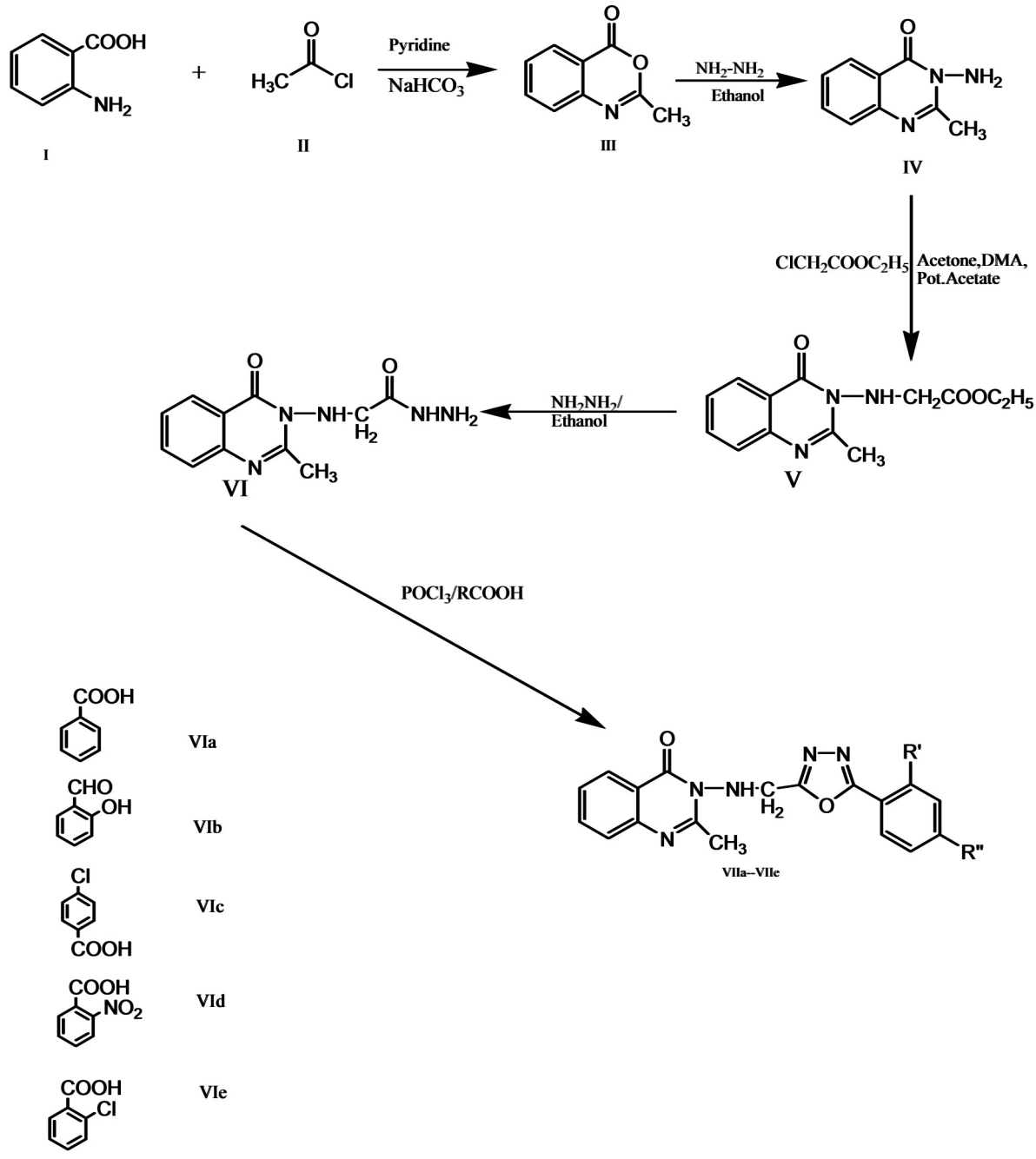
Analgesic Activity

Acetic acid induced writhing method:

The writhing test described by Koster et al. (1959) was adopted. A total of 42 mice divided into seven groups (n=6) were used and treated as follows; group 1 served as control and received vehicle alone (3% V/V tween-80 10 ml/kg) p.o., groups 2to 6 received 200 mg/kg p.o. of the synthesized compounds like VIIa, VIIb, VIIc, VIId and VIIe respectively, while group 7 received 100 mg/kg of acetyl salicylic acid (standard drug) p.o. 10 ml/kg of 0.7% aqueous solution of acetic acid were given to all mice i.p. 30 min later. Each mouse was placed in a transparent observation cage and abdominal constriction resulting from injection of acetic acid for the period of 20 minutes was counted. Results were presented as percent inhibition of analgesia, calculated as the reduction in the number of writhes between control animals and those pre-treated with

SCHEME

Fig. 1: Schematic representation for synthesizing compound VII (a-e)



either the synthesized compounds or acetyl salicylic acid [8] .

Hot plate method:

Experiments were carried out according to method described by Adzu et al., (2001). Mice that showed nociceptive responses within 20s when placed on hot plate maintained at 55 $\pm$ 0.5^{0}c were selected and grouped into seven groups of (n=6).Group 1 served as control and received vehicle alone (3% V/V tween-80 10 ml/kg) p.o: groups 2-6 received 200mg/kg p.o. of the synthesized compounds like VIIa, VIIb, VIIc, VIId and VIIe while group 7 received 100mg/kg of acetyl salicylic acid p.o. Each mouse was placed singly on the hot plate and the latency to exhibit thermal stimulus were determined at 0min, 30min, 60min and 120min before and after the treatment. Licking of paws and jumping were the parameters evaluated s the thermal stimulus. Sixty seconds was taken as the cut-off time to

avoid mouse tissue damage. Analgesic activity was expressed as mean percent Maximal effect calculated as % MPE = Post-drug latency—Pre-drug latency/cut-off time-Pre-drug latency [9].

Anti-inflammatory activity

Animals

Male albino Wister rats weighing 200–250g, supplied by Ms. B.N. Ghosh & Co., Calcutta, India, were placed in cages with wire-net floors ina controlled room temperature 29°C, relative Humidity 60–70% and provided with food and water ad libitum. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. All studies were carried out by using six rats in each group.

Carrageenan Induced Rat Paw Edema Method

Oedema was induced by sub planter injection of 0.1 ml of 1% freshly prepared suspension of carrageenan into the right hind paws of the rats off our groups of six animals each. The volume of the injected and contra-lateral paws were measured 1,3 and 5 h after induction of inflammation using a plethysmometer according to the method described by Winter et al. (1962) The test groups received the synthesized compounds (200mg/kg),the standard group received phenylbutazone (100mg/kg), and the control animals received the vehicle only alone (3% V/V tween-80, 10 ml/kg) p.o. All the treatments were given intraperitoneally 30 min prior to the injection of carrageenan except for the synthesized compounds. Increase of paw oedema thickness was calculated [10].

Dextran induced rat paw oedema

In this model oedema was induced by subplanter injection of 0.05 ml of freshly prepared 1% solution of dextran into the right hind paw of the rats. Group division of the animals and the treatment of test, standard and control animals were the same as the carrageenan model 74. For comparison purpose, the volume of oedema at various prefixed time intervals was measured. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percen tage reduction} = \frac{Vo - V_t}{Vo} \times 100$$

Where, Vo = Volume of the paw of control at time‘t’.

Vt = Volume of the paw of drug treated at time‘t’. From the data obtained, the mean oedema volume and percentage reduction in oedema was calculated. The results are expressed as mean ± S.E.M. Dennett’s t-test was used to verify the statistical significance at $p<0.05$ between the treated and control groups [11, 12].

RESULTS AND DISCUSSION

The compounds synthesized using the above scheme was subjected for TLC, elemental analysis, spectral studies like MASS, HNMR, and FTIR spectral studies and their results are discussed below. The melting point and the percentage yield of the compounds VII (a-e) are represented in Table No.1.Thin layer chromatography of the compounds showed single spot with the R*f* value of 0.69, 0.44, 0.73, 0.68 and 0.72 respectively for compounds VII (a-e).

Spectral datas of 3-((5-phenyl-1, 3, 4-oxadiazol-2-yl) methylamino)-2-methylquinazolin-4-(3H)-one VIIa:

The compound VII (a) synthesized using the abovementioned procedure showed characteristic peaks for FTIR at 3402cm-1(NH – Str 2° amine), 3044cm-1 (Ar CH-str), 1687 cm-1(C==O str),1590 (C=N-str) ,1183(C-O-C-str) HNMRδ 2.6 (3H, S, CH3), δ 5.21(2H, S, CH2), δ 7.4-8.3(9H, M, Ar-H), δ 7.1 (1H, S, N-H). The molecular ion peak M+ was obtained at 333.

Spectral data of 3-((5-phenylhydroxyl-1,3,4-oxadiazol-2-yl)methyl- amino)-2-methyl quinazolin-4(3H)-one (VIIb):

The compound VII (b) synthesized using the above mentioned procedure showed characteristic peaks for FTIR at 3575cm-1(OH –str), 3405(NH – Str 2°amine), 1700 cm-1 (C==O str), 3120 (CH – Str), 1107(C-O-C-str),1593 (C=N-str)

HNMR values are

δ 2.6 (3H, S, CH3), δ 4.95 (2H, S, CH2), δ 7.1-8.1(8H, M, Ar-H), δ 6.8 (1H, S, N-H), δ 5.4 (1H, S, O-H). The mass spectrum showed the M+ at 367 which confirms the molecular weight as 349.

Spectral data of 3-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl amino)-2-methylquinazolin-4(3H)-one (VII c):

Compound VI c showed FT-IR characteristicpeaks at 3425 cm-1(2° amine N-H Str), 1695 cm-1(C=0Str), 3043 (CH – Str), 1123(C-O-C-str), 1600 (C=N-str), 755(C-Cl str). . HNMR peaks at δ 2.5 (3H, S, CH3), δ 5.1 (2H, S, CH2), δ 7.0-8.0(8H, M, Ar-H), δ 6.8 (1H, S, N-H), δ 10.1 (1H, S, N-H).

Spectral data of 3-((5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3H)-one (VIId):

The FTIR spectrum of compound VIId showed characteristic peaks at 3413cm-1 (2° amine N-H Str) , 1681 cm-1(C=0Str), 3154 (CH – Str), 1116(C-O-C-str),1601(C=N-str),912(C-NO_2 str). HNMR peaks at δ 2.8 (3H, S, CH3), δ4.8 (2H, S, CH2), δ 7.1-7.9 (8H,M, Ar-H), and δ 6.7 (1H, S,NH).

Spectral data of 3-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methylquinazolin-4(3H)-one(VIIe).

The FTIR spectra for compound VII e showed3410cm-

1(2° amine N-H Str),3065cm-1 (Ar-CH Str), 1680 cm-1(C=0Str), 1168(C-O-C-str),1595(C=N-str),757(C-Clstr). HNMR peaks at δ 2.5 (3H, S, CH3), δ5.2 (2H, S, CH2), δ 6.5-7.3 (8H, M, Ar-H), and δ 6.9. (1H, S, NH).The above spectral values confirm the structure of the synthesized compounds and the mass spectral values also corresponds to their calculated molecular weight.

Pharmacological activity

The above mentioned compounds were subjected for various pharmacological activities and the data's of the compounds are discussed here.

Acute toxicity studies

There were no deaths of rat's upto 3000mg/kgb.w. of all the synthesized compounds VIIa, VIIb, VIIc, VIId & VIIe within short and long term out come of the limit dose test up and down procedure. However, showed some behavioral signs of toxicity include irritation, restlessness, tachypnoea, anorexia, bilateral narrowing of the eyelids and abnormal posture (which was characterised by tugging of the head in-between the hind limbs) at 3000mg/kg b.w. The LD50 was calculated to be greater than 3000mg/kg/ b.w. oral route. According to Clarke and Clarke, (1997) substances with LD50 of 1000mg/kg bodyweight/oral route are regarded as being safe or of low toxicity[23]. In the present study, acute toxicity study of all the synthesized compounds VII a, VII b, VII c, VII d & VII e showed that no mortality of rats occurred, at a limit dose of 3000 mg/ kg and5mg/kg body weight given orally. This is an indication that the all the synthesized compound shave low acute toxicity when administered orally.

Analgesic Activity

Analgesic activity of all the compounds were carried out using acetic acid induced writhing and hot-plate latency in mice model. The results of which are presented below.

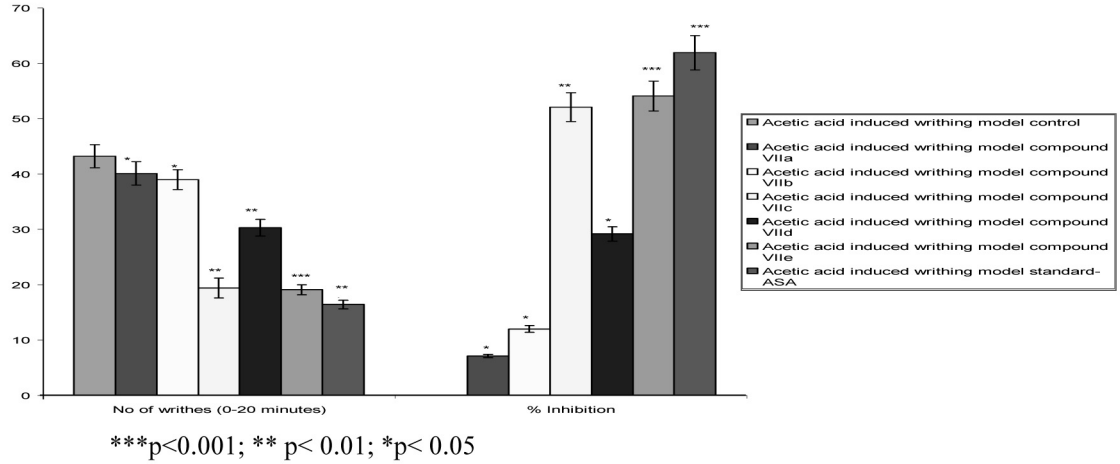
Acetic Acid Induced writhing method

The result of the acetic acid induced writhing method was represented in Fig.2. The % inhibitions were 8.63%, 42.47%, 31.19%, 57.52%and 11.7% respectively for

Table - 1:

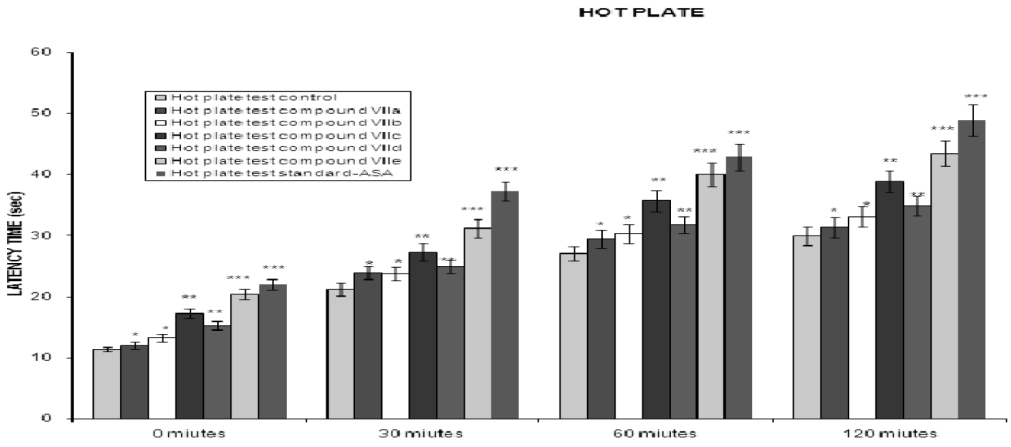
The melting point and the percentage yield of the compounds VII (a-e).

Compound No	Molecular formula	R1	R2	Molecular weight	Percentage % yield	Melting Point °C
VIIa	$C_{18}H_{15}N_5O_2$	H	—-	333.12	65%	201°c
VIIb	$C_{18}H_{15}N_5O_3$	OH	—-	349.34	59%	640°c
VIIc	$C_{18}H_{14}ClN_5O_2$	Cl	—-	367.79	51%	571°c
VIId	$C_{18}H_{13}N_6O_4$	NO_2	—-	378.34	65%	654°c
VIIe	$C_{18}H_{14}ClN_5O_2$	—-	Cl	367.79	58%	580°c



***p<0.001; ** p< 0.01; *p< 0.05

Fig.2. Graphical representation of effect of compounds VIIa , VIIb, VIIc, VIId, VIIe and standard drug in acetic acid-induced writhing test on mice



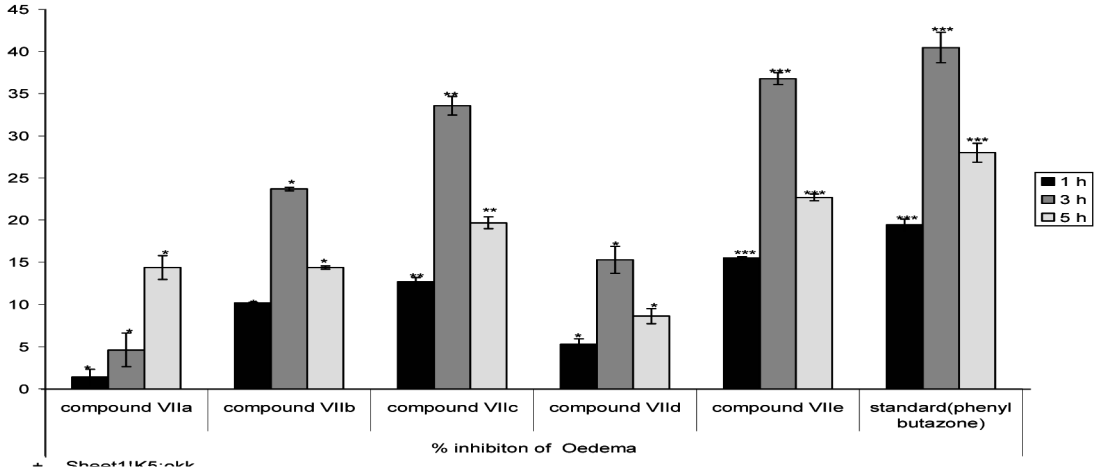
***p<0.001; ** p< 0.01; *p< 0.05

Fig.3 : Graphical representation of effect of the compounds VIIa, VIIb , VIIc, VIId, VIIe and standard drug in Hot-plate latency test on mice

compound VIa, compound VII b, compound VII c, compound VII d and compound VII e respectively with significance value less than 0.05. Compound VII c, and compound VIIe inhibited the writhing response almost to the same degree as Aspirin (61.97% inhibition) at 100mg/kg. The results suggests that the synthesized quinazolin-4(3H)-ones derivatives linked with oxadiazoles derivatives significantly inhibited the writhing response induced by acetic acid in mice, which suggested that the these compounds displayed central (Through writhing test) analgesic effects. The compounds also showed inhibitory effects on writhing response induced by acetic acid, which suggested that the quinazolin-4(3H)-ones derivatives linked with oxadiazoles acted like non-steroidal anti inflammatory drugs such as acetylsalicylic acid and the analgesic effect may attribute to its anti inflammatory action.

Hot-plate latency test in mice

The result of the Hot-plate method was represented in Fig.3. The percentage protections of the animal by the compounds from the thermal stimuli were comparable to that of Aspirin. The latencies were found to be 29.88, 43.52, 33.18, 38.63 and 34.81 for compound VIIa, compound VIIb, compound VIIc, compound VIId and compound VIIe respectively. Compound VIIc& VIIe showed the latency time almost to the same degree as aspirin (48.91%).The results showed that the compounds significantly increased the pain threshold to hot plate in Mice, which suggested that the extract displayed peripheral analgesic effect. Compounds VIIc & VIIe showed maximum % protection in hot plate test, which suggested that, the compound acted like non-steroidal anti-inflammatory drugs such as acetylsalicylic acid and the analgesic effect, may



***p<0.001; ** p< 0.01; *p< 0.05

Fig.4: Graphical representation of Percentage Inhibition of the oedema by compounds VIIc &VIIe and standard drug in Carrageenin induced paw-oedema method

attribute to its anti-inflammatory action. Thus based on the above two models it could be concluded that the compounds VIIc and VIIe posses potent analgesic activity than other compounds and was in par with the analgesic activity of the standard drug.

Anti-inflammatory activity

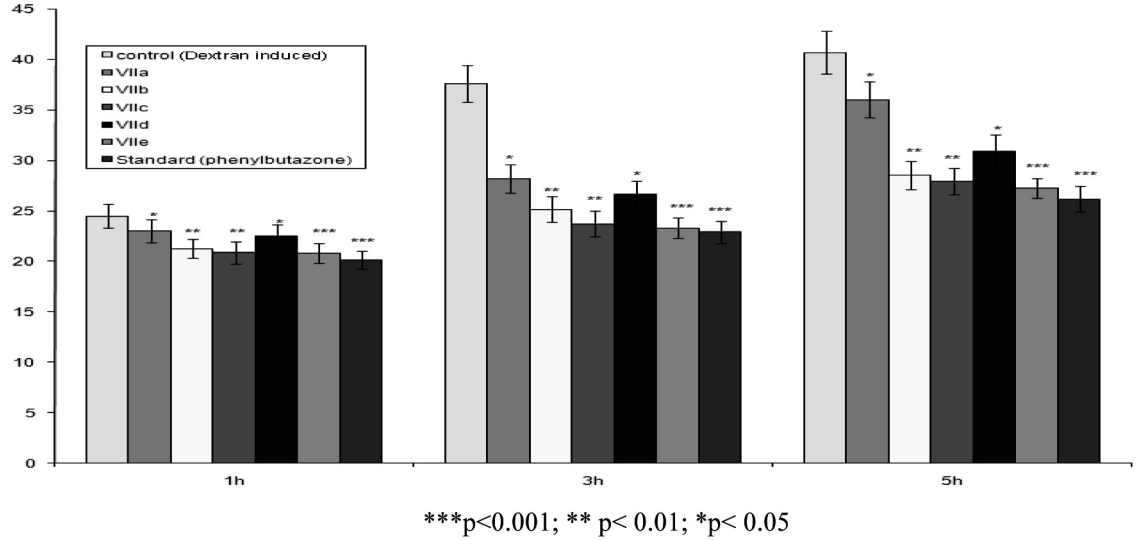
The anti-inflammatory activity was performed only for compounds VII c and VII e based on the results of analgesic activity. The anti inflammatory activity was performed using carragennan induced paw oedema method and dextran induced oedema method, and the results are discussed below.

Carrageenin Induced Paw-Oedema Method

The results of the anti-inflammatory effect of the100mg/kg of compounds VIIc&VIIe on carragenin induced oedema in rat's right hind paws are presented in Fig.4. There was a gradual increase in oedema paw volume of rats in the control (carrageenan treated). However, in the test groups, treated with compound VIIc and compound VIIe both showed a significant reduction in the oedema paw volume. The significant anti inflammatory effect induced by compounds appeared at 1h and progressively increased. The compounds VIIc & VIIe showed a maximum inhibition of 33.58% & 36.79% respectively at 3rdhour. The anti-inflammatory effect induced by Phenyl butazone progressively increased and reached a maximum (40.49%) at 3rd hour.

Dextran Induced Oedema Method

The results of the anti-inflammatory effect of the100mg/kg of compounds VIIc & VIIe on dextran induced oedema in rat's right hind paws are presented in Fig.4. There was a gradual increase in oedema paw volume of rats in the control (dextran treated). However, in the test groups, treated with compound VIIc and compound VIIe both showed a significant reduction in the oedema paw volume. The significant anti inflammatory effect induced by compounds appeared at 1h and progressively increased. The compounds VIIc & VIIe showed a maximum inhibition of 33.01% & 31.32% respectively at 3rdhour. The anti-inflammatory effect induced by Phenyl butazone progressively increased and reached a maximum (35.63%) at 3rd hour. The present study establishes the anti-inflammatory activity of the compound VIIc and VIIe. The compounds effectively suppressed the inflammation produced by HEM. It is evident that carrageeenan induced oedema is commonly used as an experimental animal model of acute inflammation and it is believed to be biphasic of which the first phase is mediated by release of histamine and serotonin in the early phase followed by kinin release and then by prostaglandin in the later phase. The effect of synthesized compounds VIIc & VIIe on the acute phase of inflammation was observedin the dextran-induced paw oedema test. Dextran induced rat paw oedema is a suitable experimental animal model for evaluating the anti-inflammatory effect of chemical products and it is believed to be biphasic (Winter et al., 1962). The first phase (1 h) involves the release of histamine and serotoninand the second phase (over 1 h) is due to the release of prostaglandin-like substances. Based onthis, the second phase may be explained by an inhibition of cyclooxygenase. Thus it can be said that compounds VIIc & VIIe reduced the oedema produced by dextran which is known to be mediated by both histamine and serotonin. 100 mg/kg doses of synthesized compounds VIIc & VIIe showed anti-inflammatory effects at all hours after dextran injection. But the most significant anti-inflammatory response occurred at the third hour, reduction in paw oedema by, 31.32% & 33.01 respectively for compound VIIc & VIIe. The standard (phenylbutazone) showed a maximum response at third hour and % inhibition is recorded to be 40.49%



***p<0.001; ** p< 0.01; *p< 0.05

Fig.5 : Graphical representation of Percentage Inhibition of the oedema by compounds VIIc &VIIe and standard drug in Dextran induced paw-oedema method.

Conclusion

As per the results of the present study, it could be concluded that, the presence of phenyl group at the1st position of the ***2-(4-oxo-2-methylquinazolin-3(4H)-yl-amino) acetohydrazide*** nucleus treated with o-chloro benzoic acid in $POCl_3$ through oxadiazole linked with at the 5thposition may be contributing to the anti-inflammatory effect. The presence of methyl group at the 3rd position of the pyrazolinone ring in compound VIIc may be enhancing the activity hence it is more effective over VIIe. Furthermore works has to be carried out on the toxicological profile and to optimize the structure of this moiety for more potent, safer and effective analgesic and anti-inflammatory drug.

Acknowledgement

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A Study on Phytochemical and In-vitro Anthelmintic Assay on Fruits of *Basella rubra Linn*

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ABSTRACT

The present study was undertaken to evaluate phytochemical and anthelmintic activity of crude macerated extracts of fresh fruits of *Basella rubra Linn.* fam. *Basellaceae.* Crude macerated extract of methanolic (MME), hydro alcohol (HAE) and 1 hour macerated fresh fruit methanolic (ME) were used for the phytochemical and anthelmintic activity. The phytochemical investigation reveals the presence of tannins, flavnoids, saponins, terpenoids and reducing sugars. In-vitro anthelmintic studies was carried out using *Pheretima posthuma* (Indian earth worm) as test worms, various concentrations (100, 200, 300, and 400 µg/ml) of crude extract, were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Albendazole used as standard reference and distilled water as control. The paralytic action and death rate of both extract of MME and ME showed better results compared to HAE. Further studies are in process to isolate the active principle/s responsible for the activity.

Key words: *Basella rubra Linn.,* Albendazole, Pheretima posthuma, anthelmintic.

Introduction

It is the whole plant of *Basella rubra Linn.* belonging to the family Basellaceae or Malabar spinach (also Phooi leaf, Red vine spinach, Creeping spinach, Climbing spinach) is a perennial vine found in the tropics where it is widely used as a leaf vegetable. *Basella rubra* is also known as Basella *alba,* it is a fast-growing, soft-stemmed vine, reaching 10 m in length. Its thick, semi-succulent, heart-shaped leaves have a mild flavor and mucilaginous texture. Leaves are broadly ovate and pointed at apex young stems and leaves are markedly fleshy [1]. The stem of the cultivar *Basella rubra* 'Rubra' is reddish-purple. *Basella rubra* grows well under full sunlight in hot, humid climates and in areas lower than 500 m above sea level. Growth is slow in low temperatures resulting in low yields. Flowering is induced during the short-day months of November to February. Leaves contain [2] , 275 Calories per 100g Water: 0% ,Protein: 20g; Fat: 3.5g; Carbohydrate: 54g; Fiber: 9g; Ash: 19g; Minerals - Calcium: 3000mg; Phosphorus: 0mg; Iron: 0mg; Magnesium: 0mg; Sodium: 0mg; Potassium: 0mg; Zinc: 0mg; Vitamins - A: 50mg; Thiamine (B1): 0.7mg; Riboflavin (B2): 1.8mg; Niacin: 7.5mg; B6: 0mg; C: 1200mg. Major components identified from volatile oil were 1-methoxypropane, (*Z*)-3-hexen-l-ol, 3-methoxyphenyl acetate, acetophenone, 4-vinylguaiacol, isophytol, and phytol. The major headspace components were ethyl acetate, benzene, 3-heptanone, 2-heptene, ethyl benzene, *o*-xylene, and limonene [3]. Basella saponins A, B, C, and D, oleanane-type triterpenes oligoglycosides, together with betavulgaroside 1, spinacoside C, and momordins IIb and IIc, from fresh aerial parts [4]. Its leaves are known for being rich in â-carotene and vitamin A, and fruits are purplish-red and fleshy. Their pigments are soluble in water and offer great tinctural power, which makes them a potential source of natural dye. Anthocyanins are glycosilates from anthocyanidins, the nucleus of which is the structure of a 4'-hidroxifl avilum ion. All anthocyanins are composed of two or three parts: the basic structure, which is aglycone (anthocyanidin), sugar and frequently an acyl group [5]. The most common order of sugar frequency is: glucose, rhamnose, xylose, galactose, arabinose and fructose. However, in some cases anthocyanidins can be glycosylated. The glicosylation can occur in positions 3, 5 or 7, as the most common ones are linked to hydroxyls in positions 3 and 5 and the least common to hydroxyl in 7[6]. Basella *rubra* fruits revealed the presence of betanidin

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monoglucoside as the major betacyanin and its 4-coumaroyl and feruloyl derivatives as minor components. Co-chromatography (TLC, HPLC) with betacyanin standards identified the betacyanins as gomphrenin I (15*S*-betanidin 6-*O*-â-glucoside), gomphrenin II (15*S*-betanidin 6-*O*-[62 -*O*-(4-coumaroyl)-â-glucoside]) together with small amounts of the respective *R* forms (isogomphrenin I and II) and gomphrenin III (15*S*-betanidin 6-*O*-[62 -*O*-feruloyl-â-glucoside]). Roots are employed as rubefacient. Poultice of leaves used to reduce local swelling. Sap is applied to acne eruptions to reduce inflammation. Decoction of leaves used for its mild laxative effects. Pulped leaves applied to boils and ulcers to hasten suppuration sugared juice of leaves useful for catarrhal afflictions. Leaf-juice, mixed with butter, is soothing and cooling when applied to burns and scalds. This is a very popular medicine used by Ayurvadic healers for hemorrhages, skin diseases, sexual weakness, ulcers and as laxative in children and pregnant women. In Nigeria, it is used for fertility enhancement in women. The purpled/bruised, on account of the presence of mucilage, are used as poultice. The juice of leaves is prescribed in cases of constipation [1], particularly in children and in pregnant women and in utricaria. The present study is to investigate anthelmintic property of fruits of *Basella rubra Linn.*

Materials And Methods

Collection and Authentication:

Plant materials

The fresh Fruits of *Basella rubra Linn.* were, collected from local area of Anantapur in the month of 1st week of March. The plant was identified and authenticated by Dr. Prasad, Professor, department of botany, S. K. University, Anantapur, Andhra Pradesh.

Animals

Indian adult earth worm (*Pheretima posthuma*) was collected and identified from Dr. Philip Department of Zoology, S K University, Anantapur and Department of Soil and fertilization, Anantapur, A.P.

Drugs and chemicals

The following drugs and chemicals were used. Drugs: Albendazole (BANDY, Mankind Pharma Ltd., New Delhi), Chemicals: Methanol A.R. (Finar chemicals, Pune), DMSO A.R. (SD Fine Ltd, Mumbai), Sodium chloride (Finar chemicals, Pune), Distilled water.

Extraction of fruits of *Basella rubra Linn:*

Hydro-alcoholic extract (HAE) and Methanolic extract (MME) (7 days maceration): Fruits were crushed using mortar and pestle. Crushed material of Fruits of *Basella rubra* (200gm) was kept for maceration with 50ml of distilled water and 50ml of methanol and methanol for seven days. The extract was filtered by using muslin cloth and also, using Whatmann filter paper no.1 the extract was concentrated keeping on water bath, then dried and weighed. Both the extract was dark pinkish brown in colour and sweeties odor. The percentage yield of HAE and MME were 0.635%w/w and 1.204%w/w respectively.

***Methanolic extract* (maceration for one hour):**

Fresh fruits were crushed using mortar and pestle .Crushed material of Fruits of *Basella rubra* (200gm) was kept for maceration with 100ml of methanol for 1 hour. The extract was filtered by using Whatmann filter paper no.1 and also using muslin cloth, the extract was concentrated keeping on water bath, then dried and weighed. The ME extract was dark pinkish brown in colour and sweeties odor, the percentage yield was 1.33%w/w respectively.

Screening Methods

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by standard methods [7]. The screening covered mainly alkaloids, glycosides, sterols, terpenes, flavanoids, saponin, tannins, protein and reducing sugar. The presences of phytoconstituents are reported in Table -1.

Table-1:
Photochemical screening of aqueous and methanolic extracts of *fruits of Basella rubra Linn*

Sl. No.	Extract of *Basella rubra*	Terpe noids	Alkaloid	Reducing sugar	Tannins	Gums	Glycoside	Flavo noids	Saponins	Proteins
1	*Hydroalcoholic (7days macerated)*	+	-	+	+	-	-	+	+	-
2	*Methanolic (7days macerated)*	+	-	+	+	-	-	+	+	-
3	*Methanolic (1 hr macerated)*	+	-	+	+	-	-	+	+	-

(+) indicate presence (-) indicate absence.

In-vitro Anthelmintic Assay

The Anthelmintic assay was carried out as per the method of Ajaiyeoba et al [8]. The assay was performed in vitro using adult Indian earthworm and *Pheretima posthuma* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation Anthelmintic activity [9]. Earthworms (Pheretima posthuma) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. In-vitro bio assay was carried out using, five groups of six earthworms worms i.e. *Pheretima Posthuma* approximately equal size (6-8 cm) were released in to 25 ml of solutions of Albendazole, and various concentration of crude extracts (HAE, MME, ME) in the range of 100, 200, 300, and 400 µg/ml were prepared using with distilled water in Petri dish. Albendazole was used as reference standard and distilled water as control. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously colour of the body nor when dipped in warm water (50^0 C).

Results and Discussion

Preliminary phytochemical screening for the extracts HAE, MME and ME reveals the presence of tannins, flavnoids, saponines, terpenoids, and reducing sugars are tabulated in the table no 1. Tannins are proven to show the anthelmintic activities [10]; chemically these tannins are polyphenolic compound [11], and reported that some synthetic phenolic compounds interfere with energy of helminth parasites by uncoupling oxidative phosphorylation [12]. In- vitro anthelmintic results shown in Table-2, the predominant effect of Albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [13-15]. From the table it is evident that various concentration (100, 200, 300, and 400 µg/ml) of fruit extracts of *Basella rubra Linn.* showed paralytic and death time as the concentration increasing. Paralytic effect (P) of HAE extract showed good activity when compared with the MME and ME, 17.33±0.49, 15±0.57, 13.83±0.60, and 12.83±0.60 respectively, but the death time (D), were slow when we compare with other two extracts of MME and ME since the paralytic time was slow but both the methanolic extract showed faster death time than HAE extract respectively.

Conclusion

It can be concluded that the present report confirms that the methanolic extract of *Basella rubra Linn.* shows potent anthelmintic activity. The results of paralysis time and death time of methanolic extract (MME and ME) were showing greater activity at different concentration than standard albendazole. Phytochemical screening reveals that the presence of tannins which are reported for their anthelmintic activity by various researchers in natural products. Future plan of work includes isolated, purification

Table-2:
In-vitro Anthelmintic activity of hydro-alcoholic and methanolic extracts of fruits of *Basella rubra Linn.*

SL. No	Treatment	Dose (µg/ml)	Time Taken For Paralysis (Min) MEAN±SEM	Time Taken For Death (Min)MEAN±SEM
1	Control (Distilled Water)	——	——	——
2	Standard (Albendazole. Tab)	360	44.5±0.42	89.5±0.42
3	Hydroalcholic Extract	100	17.33±0.49	80.6±0.55
	(7 days maceration)	200	15±0.57	73.6±0.71
		300	13.83±0.60	60.8±0.60
		400	12.83±0.60	48.16±0.65
4	Methanolic Extract	100	45.16±0.60	86.66±0.49
	(7 days maceration)	200	34.83±0.60	72.5±0.88
		300	21.8±0.47	38.5±0.61
		400	13±0.57	19.8±0.60
5	Methanolic Extract	100	33.6±0.66	86±0.57
	(1 hour maceration)	200	24±0.57	69.5±0.42
		300	14.16±0.60	46±0.57
		400	17.33±0.49	22.0±0.57

Results expressed as Mean ± SEM from 6 observations for paralysis and death time of earthworm (*Pheretima posthuma)*

and characterization of tannins from fresh fruits of *Basella rubra Linn.*

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Synthesis and Analgesic Activity of Some New Semisynthetic Derivatives of Carvone

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ABSTRACT

In the present study various semi synthetic derivatives of Carvone was synthesized and their anti-inflammatory activity was evaluated. The anti-inflammatory activity was performed on male Wistar rats (200-250g) using carrageenan induced paw edema model. Diclofenac sodium was used as standard. The results of the anti-inflammatory study indicates that compound III and compound V has good anti-inflammatory activity when compared with other derivatives and showed mild to moderate activity in comparison with the standard drug.

Key words: Carvone, Anti-Inflammatory, Semi synthetic derivatives.

Introduction

As a result of rapid development of phytochemistry and pharmacological testing methods in recent years, new plant drugs are finding their way into medicine as purified phytochemicals, rather than in the form of traditional galenical preparations.[1] These small molecules provide the source of inspiration for the majority of FDA-approved agents and continue to be one of the major sources of inspiration for drug discovery. In particular, these compounds are important in the treatment of life-threatening conditions.[2] Some of the natural drugs may not have potency to treat diseases. So there the concept of semi synthetic chemistry araised and for the first time in 1869 Brown and Fraser while working on relationship between molecular structure and biological activity, identified that N-Methyl morphine and N-Methylatropin are muscle relaxants instead their parent natural compounds, morphine is an analgesic and atropine is an mydriatic agent. Then after working on the semi synthetic compounds increased and further investigations were carried out.[3] The above concept has brought many drugs into market best example for this is Aspirin (Acetyl salicylic acid) a semi synthetic derivative of salicylic acid. The present work was designed based on this concept to synthesize some novel semisynthetic derivatives and to evaluate its possible biological activity.

Carvone, a terpenoid is a principal constituent of the caraway seed oil *(fa*m. *Carum carvi)*. Carvone is used in the food and flavor industry .Carvone is also used for air freshening products and, oils containing Carvone are used in aromatherapy and alternative medicine. Further the compound was found to be used as analgesics, antimicrobial agent, antifungal agent, and also relieves many respiratory tract infections [4-6]. The major drawback of Carvone is more volatile in nature and thus the present work was designed to synthesize various nonvolatile derivatives of Carvone and to evaluate its possible anti-inflammatory activity.

*Address for correspondence

Materials and Methods

Chemicals and reagents

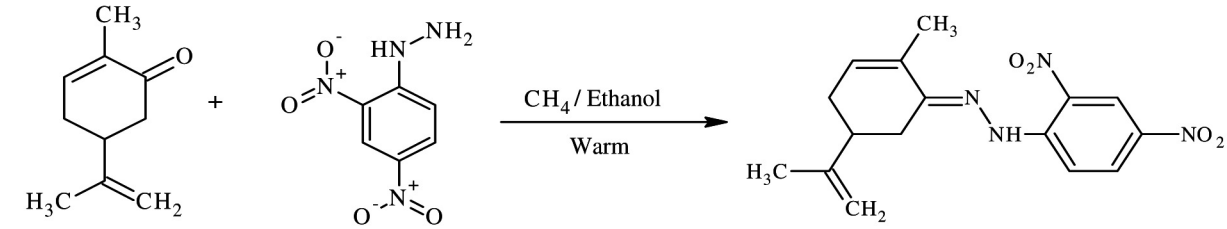
Carvone was presented as a gift sample by Director, JNTU, OTRI, Anantapur. 2, 4-DNP Sodium acetate purchased from S.D-fine chem. Ltd, Mumbai Hydrazine sulphate (IDPC-Hyderabad), Semicarbazone (Sisco Research Laboratories, Mumbai), Ethanol (Changshu Yang Yuan Chemical Ltd), O-phenyl diamine (Oxford Lab, Mumbai).

Synthesis

The scheme for the synthesis of Carvone derivatives is represented in Fig.1.

Procedure for synthesis of compound I (2E)-1-(2,4-Dinitrophenyl)-2-[2-Methyl-5-(Prop-1-en-2-yl)cyclohex-2-en-1-ylidene]Hydrazine from Carvone[7].

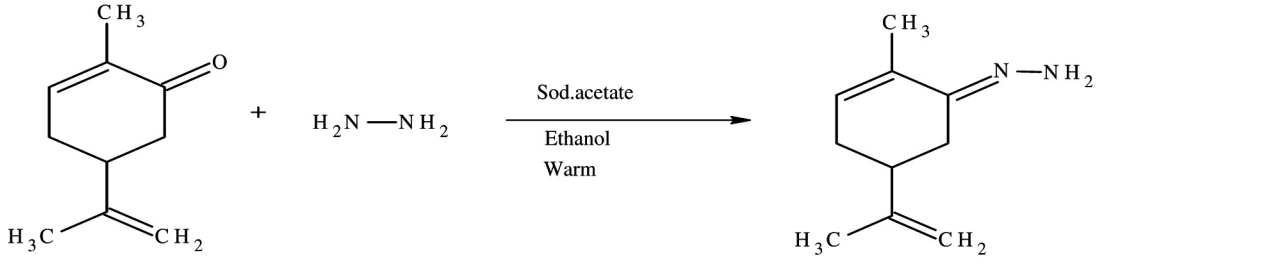
0.5gm 2,4 Dinitro phenyl hydrazine and 0.8gm of sodium acetate was dissolved in 5ml of water, and a solution of 0.4g of carvone in a little ethanol was added



Carvone

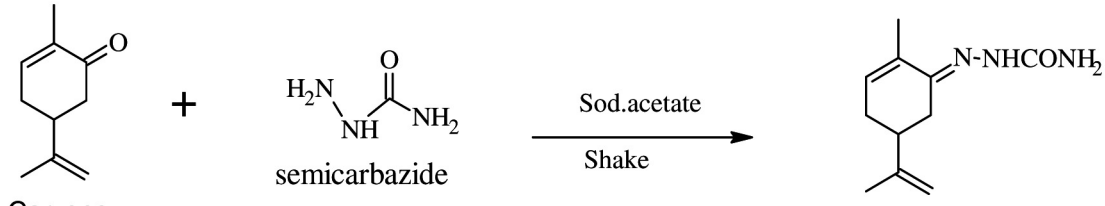
2,4 Dinitro Phenyl Hydrazine

(2E)-1-(2,4-dinitrophenyl)-2-[2-methyl-5-(prop-1 -en-2-yl)cyclohex-2-en-1-ylidene]hydrazine



Carvone

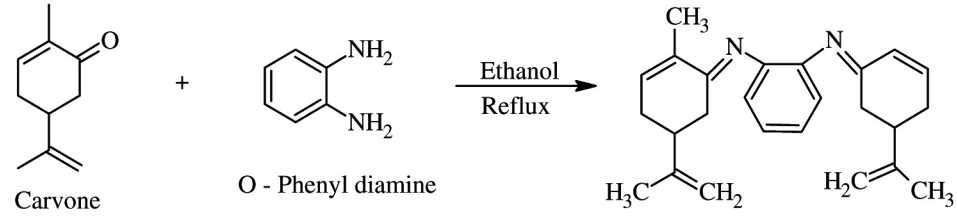
(1E)-[2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-ylidene]hydrazine



Carvone

semicarbazide

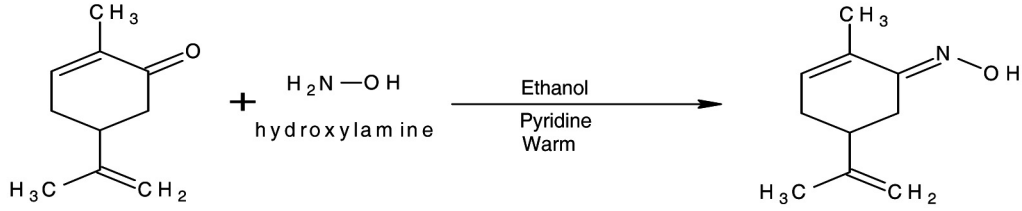
(2E)-2-[2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-ylid ene]hydrazinecarboxamide



Carvone

O - Phenyl diamine

N,N'-Bis[(1e)-2-Methyl-5-(Prop-1-En-2-Yl)Cyclohex-2-En-1-Ylidine]Benzene-1,2-Diamir



Carvone

(1*E*)-*N*-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-imine

Fig. 1 : Scheme for synthesis of Carvone derivatives

and the mixture was stirred until clear solution appears. Then warm on water bath for 10-15minutes and cooled to obtain product. The product was filtered using Buchner funnel and washed with cold water and dried. Recrystallize by using ethanol. Yield 59%, m.p. 195°C.

Spectral Data: FT-IR Spectrum of Compound I showed characteristic absorbance peak of N-H Stretching at 3438.41cm^{-1}methyl at 3321.52 cm^{-1}, C-H stretching alicyclic at 2922.53 cm^{-1}, conjugate C=C at 1644.cm^{-1},NO_2 at 1583.12 and 1329.20 cm^{-1}. ^{1}HNMR spectrum showed characteristic signals (ä ppm) at 8.87q (1H-Ar-CH), 8.33q (1H-Ar-CH), 7.0s (1H-NH), 6.98q (1H-Ar-CH), 5.5q (1H-=CH), 4.88d (1H-=CH), 4.63d (1H-=CH), 2.2q (1H-CH), 2.09-1.84m (2H-CH_2), 1.52-1.2m (2H-CH_2).The mass spectrum of the compound showed its molecular ion (M^+) peak at m/z at 330.

Procedure for synthesis of compound II (1E)-[2-Methyl-5-(Prop-1-en-2-yl) cyclohex-2-en-1-ylidene] Hydrazine from Carvone[7].

0.5gm hydrazine and 0.8gm of sodium acetate was dissolved in 5ml of water, and a solution of 0.4g carvone of in a little ethanol was added and the mixture was until clear solution appears. Then warm on water bath for 10-15minutes and cooled to obtain product. The product was filtered using Buchnaer funnel and washed with cold water, dried and recrystallized using ethanol. Yield 70.3%, m.p. 160°C.

Spectral data: FT-IR Spectrum of Compound II showed characteristic absorbance peak of N-H stretching at 3377.46cm^{-1}, C-H cyclic stretching at 3101.81 cm^{-1}, C=C at 1519.80 cm^{-1}. ^{1}HNMR spectrum showed characteristic signals (ä ppm) at 7.0s (2H-NH_2), 5.5q (1H-=CH), 4.88d (1H-=CH), 4.63d (1H-=CH), 2.2q (1H-CH), 2.09-1.84m (2H-CH_2), 1.71d (6H-CH_3), 1.5-1.2m (2H-CH_2).The mass spectrum of the compound showed its molecular ion (M^+) peak at m/z at 164.

Procedure for synthesis of compound III (2E)-2-[2-Methyl-5-(Prop-1-en-2-yl)Cyclohex-2-en-1-Ylidene]Hydrazine Carboxamide from Carvone[7].

1gm of semi carbazine and 1.5gm of crystallized sodium acetate was dissolved in 8-10ml of water to this mixture, 0.5gm of the carvone was added and shaken for a while, then minute quantity of alcohol was added and continued shaking until a clear solution was obtained. Then the mixture was allowed to stand for crystallization of semicarbazone. The crystals were filtered and washed with cold water and recrystalized from dilute ethanol. Yield 53.3%, m.p. 95°C.

Spectral data: FT-IR Spectrum of Compound showed characteristic absorbance peak of N-H stretching at 3456.58 cm^{-}1, C-H stretching at 3404.97 cm^{-1}, C-H bending methyl at 3206.72 cm^{-1},CH stretching at 2924.28 cm^{-1}, C=0 twisting at 1688.85 cm^{-1}, C=C ring stretching at b1572.92 cm^{-1}, CH bending acyclic at 1379.08 cm^{-1}. ^{1}HNMR spectrum showed characteristic signals (ä ppm) at 7.0s (1H-NH), 6.0s (2H-NH_2), 5.5d (1H-=CH), 4.88d (1H-=CH), 4.63d (1H-=CH), 2.2q (1H-CH), 2.09-1.85m (2H-CH_2), 1.71d (6H-CH_3), 1.52-1.2m (2H-CH2).The mass spectrum of the compound showed its molecular ion (M^+) peak at m/z at 207.

Procedure for synthesis of compound IV N,N'-Bis[(1E)-2-Methyl-5-(Prop-1-en-2-yl)cyclohex-2-En-1-ylidine]Benzene-1,2-Diamine from Carvone[8].

Carvone and *o*-phenylenediamine (0.0050mol) in ethanol was taken in a 250ml RBF and refluxed for 3 hours. Allow the mixture to cool. The precipitated product was filtered and recrystallized from the ethanol and dried in vacuum over calcium chloride. Yield 57%, m.p. 163°C.

Spectral data: FT-IR Spectrum of Compound showed characteristic absorbance peak of N-H stretching at 3439.17 cm^{-1}, C-H stretching and methyl at 2810.01 cm^{-1}, C=C ring stretching aromatic at 1581.50 cm^{-1}, C=C ring aromatic at 1497.66 cm^{-1}. ^{1}HNMR spectrum showed characteristic signals (ä ppm) at 7.3q (4H-Ar-CH), 5.7q (1H-=CH), 5.5q (1H-=CH), 4.88d (2H-=CH), 4.63d (2H-=CH), 2.2q (2H-CH), 2.09-1.84m (4H-CH_2), 1.71d (9H-CH_3), 1.5-1.2m (4H-CH_2).The mass spectrum of the compound showed its molecular ion (M^+) peak at m/z at 358.

Procedure for synthesis of compound V (1E)-n-Hydroxy-2-Methyl-5-(Prop-1-en-2-yl)cyclohex-2-en-1-Imine from Carvone[7].

Mixture of 0.5gm of carvone, 0.5gm of hydroxylamine, 5ml of ethanol and 0.5ml of pyridine was taken in a round bottom flask and refluxed on a water bath for 30minutes. Remove the ethanol by evaporation of the hot solution in a stream of air, cool. To the above mixture 5ml of water was added and kept in an ice bath and stirred until the oxime crystallizes out. The solid was filtered and washed with little water, dried and recrystalized using ethanol. Yield 59%, m.p. 195°C.

Spectral data: FT-IR Spectrum of Compound I showed characteristic absorbance peak of N-H Stretching at 3438.41cm^{-1}methyl at 3321.52 cm^{-1}, C-H stretching alicyclic at 2922.53 cm^{-1}, conjugate C=C at 1644.cm^{-1},NO_2 at 1583.12 and 1329.20 cm^{-1}. ^{1}HNMR spectrum showed characteristic signals (ä ppm) at 5.5q (1H-=CH), 4.83d (1H-=CH), 4.69d (1H-=CH), 2.2q (1H-CH), 2.09-1.84m (2H-CH_2), 2.0s (1H-OH), 1.71d (6H-CH_3), 1.5-1.2m (2H-CH_2). The mass spectrum of the compound showed its molecular ion (M^+) peak at m/z at 165.

Acute Anti-inflammatory Studies [9–11]

Carrageenan, induced rat paw edema model were used for evaluating potential of test compounds on inflammation. For each model, rats were divided in two groups (n = 6). 200-250 mg /kg of test compound and

diclofenac sodium (10 mg/kg) were administered orally one hour before the sub plantar injection of edematogenic agent. The control groups of animals were received vehicle (1 ml/kg) orally. Plethysmograph was used for measuring paw volume (mm) of rats.

Edema (T) was calculated as follows:

$T = Tt - T0$

Where Tt is the right hind paw volume (mm) at time't',

T0 is hind paw volume (mm) before sub plantar injection.

In this method, acute inflammation was produced by the subplantar administration of 0.1 ml of 1% w/v carrageenan in the right paw of the rat. The volume (mm) of the paw was measured immediately and at 1, 2, 3 and 4 hr intervals after the administration of the carrageenan. The results are tabulated in Table-1 and represented graphically in Fig.1.

Results and Discussion

The anti inflammatory activity of all the synthesized compounds was carried out using Male, Wister rats. Anti-inflammatory activity was evaluated by carrageenan induced paw edema model using the standard drug diclofenac sodium (10mg/ml) and results are presented in Table-1.The results mentioned showed good significance value with $P < 0.05$.

Compound V and III showed potent anti-inflammatory activity in par with the standard drug. The other derivatives showed moderate activity with 15 to 30 percentage reduction of inflammation when compared with the standard drug. As the carrageenan-induced paw edema model involves several chemical mediators such as prostaglandins, serotonin, histamine and bradykinin [12], thus the anti-inflammatory activity of Carvone derivatives may be due to inhibition of some of these inflammatory mediators.

Table-1:

Anti-inflammatory activity data's of semi synthetic derivatives of Carvone

Compound	*1hr*		*2hr*		*3hr*		*4hr*	
	Mean ± SD	**% red**	**Mean ± SD**	**% red**	**Mean ± SD**	**% red**	**Mean ± SD**	**% red**
Control	3.32±0.18	NA	3.47±0.19	NA	3.57±0.17	NA	3.27±0.25	NA
Standard	2.33±0.16*	29.81	2.13±0.16*	38.61	2.07±0.1*	42.01	1.83±0.13	44.03
I	3.15±0.10	7.89	3.07±0.12	12.78	2.93±0.08	18.61	2.9±0.11	15.20
II	3.02±0.07	11.69	2.90±0.08	17.61	2.80±0.12	22.22	2.68±0.11	21.63
III	2.9±0.07	15.20	2.80±0.08	20.45	2.72±0.11*	24.44	2.6±0.14*	33.97
IV	3.18±0.07	7.01	3.10±0.08	11.93	3.0±0.07	16.66	2.9±0.07*	35.20
V	2.68±0.07	21.63	2.55±0.08*	27.55	2.44±0.08*	32.22	2.3±0.08*	32.74

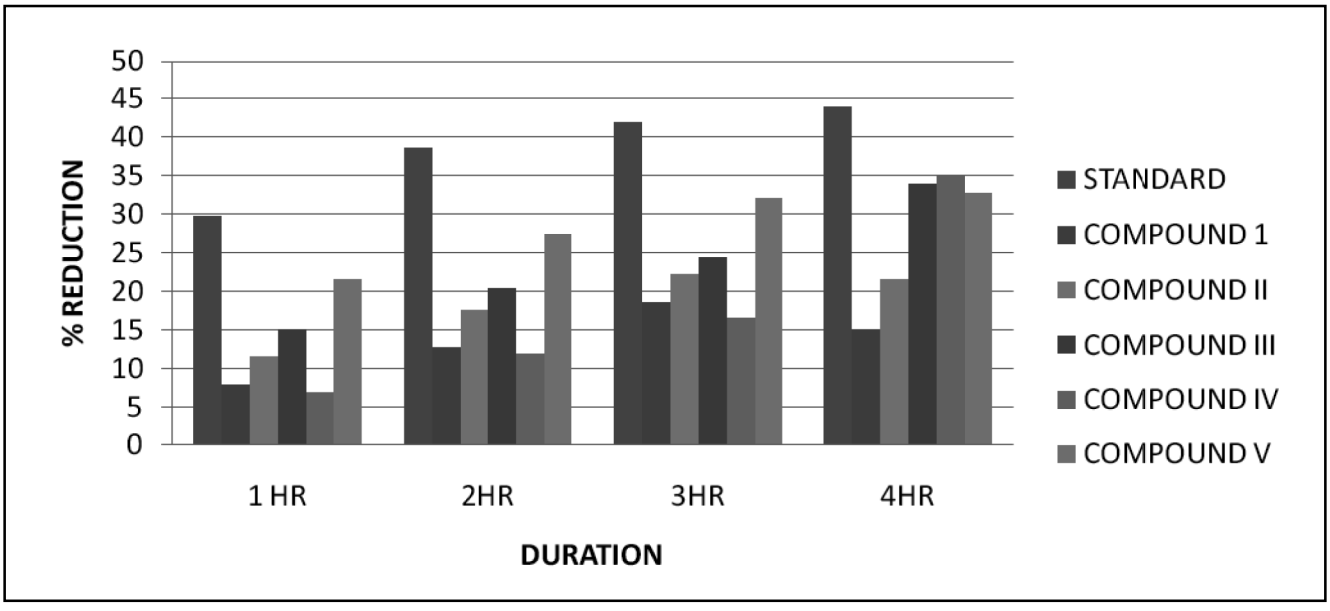


Fig. 1: Anti-inflammatory activity of Carvone Derivatives

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