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Comparative *in-vitro* free radical scavenging activity of *Murraya koenigii* leaves extract and synthesized 1, 2, 3, 4-tetrahydrocarbazole and its derivatives

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Abstract

The present study was designed to investigate the free radical scavenging activity of the chloroform extract of *Murraya koenigii* leaves and synthesized 1,2,3,4-tetrahydrocarbazole and its derivatives The chloroform extract was evaluated by TLC and its qualitative chemical investigation indicates the presence of alkaloid. Free radical scavenging activity of extract and synthesized compound was determined by DPPH free radical scavenging assay. Ascorbic acid taken as standard. Activity of extract and synthesized compounds were compared to reference compound, extract activity was lie between the activity of comp1and comp1a. Compound 1 and 1a was found to be most active among all synthesized compounds.

Key word: *Murraya koenigii*, DPPH, 1, 2, 3, 4-tetrahydrocarbazole, free radical scavenging activity.

Introduction

Murraya koenigii, commonly known as *curry* leaf or *Kari patta* in Indian accent, belonging to Family Rutaceae which exemplifies more than 150 genera and 1600 species. *Murraya Koenigii* having high values for its characteristic aroma and medicinal activity[1,-5]. It is an important export material from India as it retries good foreign return. A number of chemical constituents from every part of the plant have been extracted.[6-8] The most important chemical constituents responsible for its intense characteristic aroma are *P*-gurjunene, *P*-caryophyllene, *P*-elemene and *O*-phellandrene.[9,12] The plant contains high amount of carbazole alkaloids. *M. koenigii* is widely used in Indian cuisine for centuries and have a facile role to play in traditional medicine. Isolated carbazole alkaloids from fresh curry leaf such as mahanimbine, murrayanol and mahanine have been reported to show anti-microbial properties [13,16] .Carbazole has a wide area of biological interest such as cancer and cardiovascular disorders.It also show antioxidant, antidiabetic, antimitotic , antimicrobial [17-19], anti-vascular , antitumour , anticancer, antipsychotic, and anticonvulsant activities.

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The tetrahydrocarbazole ring system has been the structural subunit of many naturally occurring alkaloids, biologically active molecules and medicinal important synthetic analogue [20]

Material and methods

Plant material: The leaves of *Murraya Koeingii* were collected from Dehradun, U.K., India.

Extraction of carbazole from *Murraya Koeingii* by using Soxhlet apparatus (hot percolation method)

The leaves were dried in shed and reduced to coarse powder by using mechanical grinder**.** Assembly was arranged and thimble was prepared and place 10 gram of air dried powdered drug and was extracted with chloroform for 1 hour, than extract solution collected and concentrated. The process was carried out until the solvent was found to be colorless. Finally the dried extracts were collected.

The extract was subjected to preliminary qualitative chemical analysis as given below:-

Test for alkaloids:

- **Dragendroff's test (Potassium bismuth iodide solution):** Alkaloids give reddish brown precipitate with dragendroff's reagent.
- **Wagner's test (Solution of iodine in potassium iodide):** Alkaloids give reddish brown precipitate with Wagner's reagent.

- **Hager's test (Saturated solution of picric acid):** Alkaloids give yellow color precipitate with Hager's reagent.
- **Mayer's test (potassium mercuric iodide):** Alkaloids give yellow colour precipitate with Mayer's reagent

Chromatographic analysis

Thin layer chromatography: Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid.

Preparation of plates: Prepare a suspension of the coating substance, uniform layer of the suspension, 0.20 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 105°C for 30 min. At the time of use, dry the plates again, if necessary, as prescribed in the monographs**.**

Procedure: The adsorbent such as silica gel G is coated to a thickness of 0.3mm on clean TLC plate by pouring method. The plates are activated at 105°C for 30 minutes and used. The selection of mobile phase depends upon type of constituents to be analyzed. The development chamber perfectly saturated with solvent vapors. A sample spot is applied by capillary tube. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow standing at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualize

Visualization

The phrases ultra-violet light (254 nm) and ultra-violet light (365 nm). Plate was also sprayed with KMO_4 visualizing agent

R_f* Value:* Measure and record the distance of each spot from the point of its application and calculate the R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

***In-vitro* DPPH free radical scavenging activity**

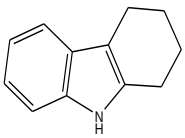
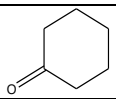
The effect of extract on DPPH radical was estimated using the method of Sooad Al-Daihan.[21]

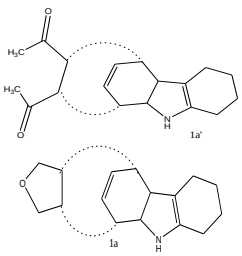
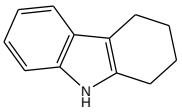
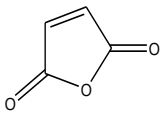
Standard DPPH solution: 0.135 mM solution, weigh 5.3mg DPPH in 100ml.

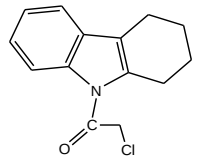
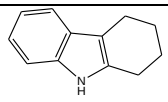
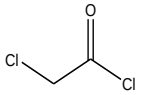
Sample stock solution: 0.1 mg/ml solution for all sample (1 mg/10 ml methanol)

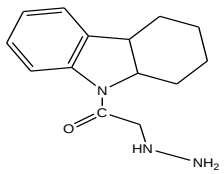
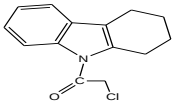
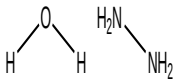
Method: A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1 ml of sample solution in methanol containing 0.02-0.1 mg of the compound. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes the absorbance of mixture was measured spectrophotometrically at 517 nm ascorbic acid was used as reference. The ability to scavange DPPH radicals was calculated by the following equation: DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$ where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract / reference.

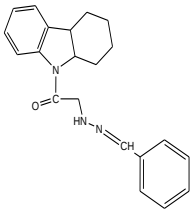
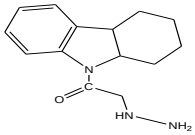
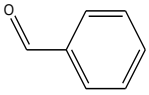
Table 1: Design of experiment for conventional method

Comp. (1)	Comp.name	Structure	wt.	Mol.wt	Eq	Mole
	Cyclohexone		8.8g, 11.31ml	84.16gm/mol	1	0.104
	Phenylhydrazone	$C_6H_5.NHNH_2$	11.24g, 10.25ml	108.14gm/mol	1	0.0804

Comp. (1a,1a')	Comp. name	Structure	wt.	Mol.wt	Eq	Mole
	1,2,3,4-tetrahydrocarbazole		4gm	171.24gm/mol	1	0.0233
	Maleic anhydride		2.28gm	98.06gm/mol	1	0.0233

Comp. (1c)	Compound	Structure	Wt	Mol. wt	Eq	Mole
	1,2,3,4-tetrahydrocarbazole		7.5gm	171.24gm/mol	1	0.0438
	Chloroacetyl chloride		4.9gm, 3.45ml	112.94gm/mol	1	0.0438

Comp.(1c′)	Comp. name	Structure	Wt	Mol.wt	Eq.	Mole
	N^9(chloroacetyl)1,2,3, 4-tetrahydrocarbazole		4gm	245.32gm/mol	1	0.0122
	Hydrazine hydrate		0.61g m, 0.64ml	50.05gm/mol	1	0.0122

Comp.(1c″)	Comp.name	Structure	wt	Mol.wt	Eq.	Mole
	N^9(hydrazinoacetyl)1,2,3,4 -tetrahydrocarbazole		3.5gm	333.43gm/mol	1	0.0105
	Benzaldehyde		1.1gm, 1.04ml	106.12gm/mol	1	0.0105

Results and Discussion

Hot percolation method: The extraction of powdered leaves material was done by using soxhlet apparatus with chloroform. Nature of extract, colour and % yield is shown in table -2

Table 2: The percentage yield of *Murraya Koenigii* Spreng. Leaves chloroform extract

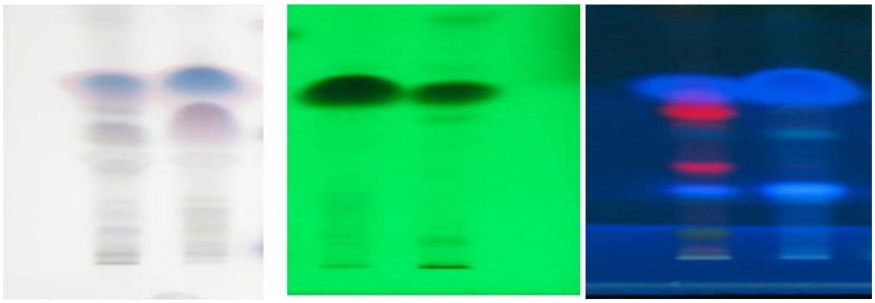
S.no	Solvent used	Nature of extract	Colour	% yield
1	Chloroform	Semi-solid	Greenish black	2.5%

The Qualitative chemical investigation of *Murraya Koenigii* Spreng. Leaves chloroform extract has indicate the presence of alkaloid table-3

Table 3:**The Qualitative chemical investigation of *Murraya Koenigii* Spreng. leaves tests for presence of alkaloids. (Chloroform extract)**

S.no	Chemical test	Chloroform extract
1.	Mayer's test	+
2.	Wagner's test	+
3.	Hager's test	+
4.	Dragendorff's test	+

Thin Layer Chromatography: TLC plate of chloroform extract of *Murraya koeingii* was developed by using n-hexane: chloroform (8:2) solvent system and visualizes by pre-derivatized method by using UV chamber and post derivatised method using $KMnO_4$ solution, Photograph of TLC of chloroform extract of *Murraya koeingii* is shown in Fig-1



A B C
$KMnO_4$ solution short wavelength UV 254 long wavelength UV 365

Fig 1: TLC plate showing spots for chloroform extract of *Murraya koeingii*

Table 4: **No. of spots and their colour by different detecting agents**

S.No	Detecting agent	No of spots	Colour
1.	**$KMnO_4$**	1I	Grey
		2	Grey
		3	Grey
		4	Grey
		5	Grey
		6	Grey
		7	Grey
		8	Pinkish
		9	Purple
		10	Pinkish blue
2.	**UV short wavelength 254**	1	Black
		2	Brown
		3	Blackish brown
3.	**UV short wavelength 356**	1	Light blue
		2	Light blue
		3	Brilliant blue
		4	Blue
		5	Dark pink
		6	Brilliant blue
		7	Grayish blue
		8	Dark pink
		9	Brilliant blue

***In-vitro* free radical scavenging activity**

The synthesized compound and chloroform extract of *Murraya Koeingii* were tested in-vitro for their free radical scavenging activity by using DPPH. The absorption of DPPH decreases due to antioxidant because of the reaction between antioxidant and free radical which results in the scavenging of free radical. Ascorbic acid taken as standard . Activity of extract and synthesized compounds were compared to reference compound, extract activity was lie between comp1and comp1a. Compound 1 and 1a was found to be most active among all synthesized compounds (**Table 5**). Absorbance of control was 0.602.

Table 5: Absorbance of Standard (Ascorbic acid) and different sample at various concentrations

Concentrations	0.02	0.04	0.06	0.08	0.1
Ascorbic acid	0.159	0.135	0.119	0.091	0.117
Extract	0.250	0.200	0.245	0.265	0.255
Comp 1a	0.312	0.295	0.300	0.311	0.301
Comp 1a′	0.212	0.182	0.200	0.215	0.198
Comp 1c	0.360	0.350	0.351	0.348	0.323
Comp 1c′	0.380	0.375	0.381	0.372	0.370
Comp 1	0.382	0.379	0.390	0.380	0.378
Comp 1c″	0.400	0.398	0.395	0.390	0.378

Table 6: **Percent inhibition of Standard (Ascorbic acid) and different synthesized compounds at various concentrations**

Concentrations	0.02	0.04	0.06	0.08	0.1
Ascorbic acid	73.6%	77.6%	80.2%	84.2%	80.6%
Extract	58.5%	66.8%.	64.3%	56.8%	57.6%
Comp 1a	48.2%	51.8%	50.2%	48.3%	50%
Comp 1a′	64.8%	69.8%	66.8%	64.3%	67.1%
Comp 1c	40.2%	41.8%	41.9%	42.2%	46.3%
Comp 1c′	36.9%	37.7%	36.7%	38.2%	38.5%
Comp 1	36.5%	37%	35.2%	36.9%	37.2%
Comp 1c″	33.6%	33.9%	34.4%	35.2%	34.2%

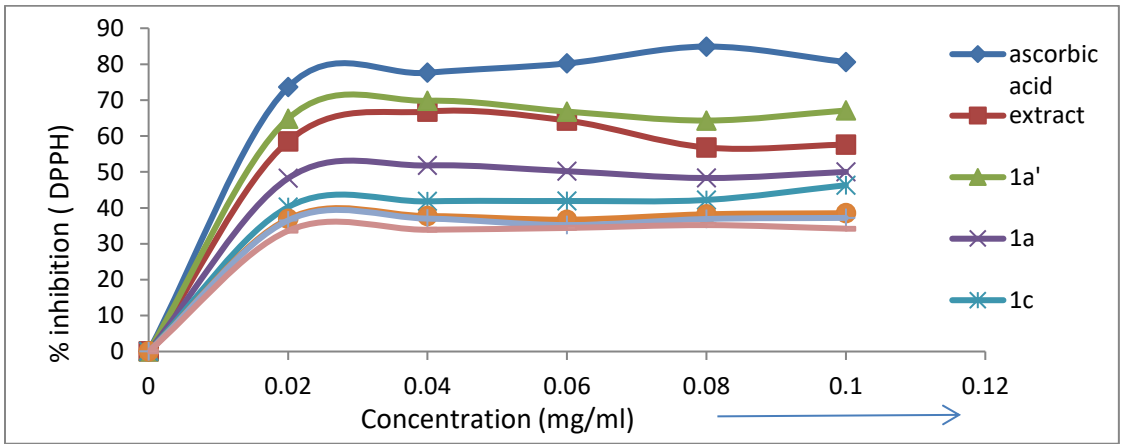


Fig 2: DPPH % free radical inhibition Vs Concentration mg/ml

Conclusion

Chloroform extract of leaves of the plant as well as the various synthesized carbazole derivatives were subjected to in-vitro antioxidant activity against the DPPH generated free radicals, result suggests that there is possible use of various synthesized compounds as well as the leaf of the plant as the newer antioxidant. As it is already known that free radicals have implicated in causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, CNS disorders etc. Thus study could be concluded as the compounds have considerable antioxidant activity. Thus, it may be concluded that the synthesized compound effectively can be further used in the treatment of above mention ailments.

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