Format of Final Report

COVER PAGE

UNVERSITY OF DELHI

INNOVATION PROJECTS 2015-16

FINAL REPORT

1. PROJECT CODE: **MH-308**

2. PROJECT TITLE: **Synthesis of Henna (Lawsonia inermis) based Scaffolds and Comparison of their Color and Antimicrobial activity.**

3. NAME OF COLLEGE/INSTITUTION: **Miranda House**

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University of Delhi

Certificate of Originality

This is to certify that the research work carried out and the final report submitted by the Project Investigators and the students of Innovation Project having Project code ***MH-308*** and title ***Synthesis of Henna (Lawsonia inermis) based Scaffolds and Comparison of their Color and Antimicrobial activity*** of ***Miranda House*** is original.

Any plagiarism/academic dishonesty reported at any stage will be our responsibility.

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Utilization Certificate

Innovation Project MH – 308 (2015-16)

Project Title : Synthesis of Henna (*Lawsonia inermis)* based Scaffolds and Comparison of their Color and Antimicrobial activity

Audited Financial Statement under Innovation Project scheme

College: Miranda House

Project Investigators: Dr Sharda Mahilkar Sonkar, Dr Anshika Lumb, Dr Monika Sharma, Dr Madhulika Johri Verma

Grant Sanctioned		(In figures) Rs 6,00,000/- (In Words) Six Lakh rupees only		
S. No.	Budget Head	Amount Sanctioned	Amount Utilised	Balance
	Equipments/Consumables	3,25,000	325000	NIL
	Travel	55,000	8070	46930
	Stipend	1,20,000	112000	8000
	Honorarium	25,000	12500	12500
	Stationery	20,000	9736	10264
	Contingency	55,000	55000	NIL
	Total amount utilized Rs. (In figures and words)	522306/- (Five Lakh Twenty Two Thousand Three hundred and six rupees only)		
	Amount remaining Rs. (In figures and words)	77694/- (Seventy Seven thousand Six Hundred and Ninty Four only)		

Certified that out of Rs. 600000/- (Six Lakh only) sanctioned to Innovation Project Code MH-308, Rs 522306/- (Five Lakh Twenty Two Thousand Three hundred and Six rupees only) has been utilized during the period of the project. The remaining amount 77694/- (Seventy Seven thousand Six Hundred and Ninty Four only) is being returned back to the University.

Signature of Project Investigators

Dr Sharda Mahilkar Sonkar Dr Anshika Lumb Dr Monika Sharma Dr Madhulika Johri Verma

Signature of Principal
Principal
Miranda House

Gupta Nandan & Associates * Chartered Accountants * New Delhi M. No. 086537

Financial Audit Clearance and Stamp of Chartered Accountant

Final Report

1. Project Title

Synthesis of Henna (Lawsoniainermis) based scaffolds and Comparison of their Color and Antimicrobial activity.

2. Project Code: MH-308

3. Abstract

The utilization of water as a solvent and lemon juice as a catalyst for the synthesis of 3,3-arylmethylenebis(2-hydroxy naphthalene-1,4-diones) by condensation reaction of aromatic aldehyde and 2-hydroxy-1,4-naphthoquinone under reflux and microwave irradiation has been studied. The green methodologies defined herein avoid severe conditions and prove to be efficient in terms of good yields, operational simplicity, easy work up and short reaction time. We also studied the variation in λ_{max} of Lawsone (2-Hydroxy-1,4-naphthoquinone) in different solution. These henna based scaffolds showed anti microbial properties which is comparable to parent compound Lawsone.

4. Introduction

Quinone cores are common in nature and play important physiological roles in animals and plants.[1] These are important in biological systems and have industrial applications as dyes or drugs.[2] Among quinones, naphthoquinones constitute an interesting class of compounds due to their biological properties, industrial applications and as intermediates in the synthesis of heterocycles.[3]Naphthoquinones have many physiological roles *e.g.*, ubiquinone, plastoquinone and K vitamins are functional constituents of biochemical systems[4,5,6]. They are usually yellow or brown in colour, and play important roles as dyes in pigmentation. In addition, they have inhibitory effect on insect larval development and sedative or toxic effect on aquatic organisms and animals. Some of the naturally occurring naphthoquinones isolated from fungi have a broad range of biological activities such as anti microbial properties, cytotoxic and phytotoxic effects, due to their interactions with the oxidative systems of cells.

2-Hydroxy-1,4-naphthoquinone (HNQ; Lawsone) is the principal natural dye (1.0-1.4%) in the leaves of henna, Lawsoniainermis. Henna has been used for more than 4000 years not

only as a hair dye, but also as a body paint and tattoo dye. Today, semi-permanent hair dyes containing Henna as well as its pure dye ingredient HNQ are widely used and have become increasingly popular due to their natural origin.[7]

Moreover, Microwave-assisted organic synthesis (MAOS) has revolutionized organic synthesis[8]. This non-classical heating technique is slowly moving from a laboratory curiosity to an established technique that is heavily used in both academia and industry[9-13]. Therefore, many academic and industrial research groups are using MAOS as a useful technique for optimization of reactions and efficient synthesis of new chemical entities.

Because of the wide utility of napthoquinone based scaffolds there has been significant impetus in developing newer synthetic routes for these structural motifs. Recent reports on the synthesis of 3,3-arylmethylenebis(2-hydroxy naphthalene-1,4-diones) employed harsh conditions, expensive catalyst and long reaction times. Consequently there is scope for developing improved and environmentally benign methodology for the synthesis of these naphthoquinone derivatives.

We report herein a new, convenient, highly efficient protocol for the synthesis of 3,3-arylmethylenebis(2-hydroxy naphthalene-1,4-diones) via condensation of aromatic aldehyde and 2-hydroxy-1,4-naphthoquinone under reflux and microwave irradiation.

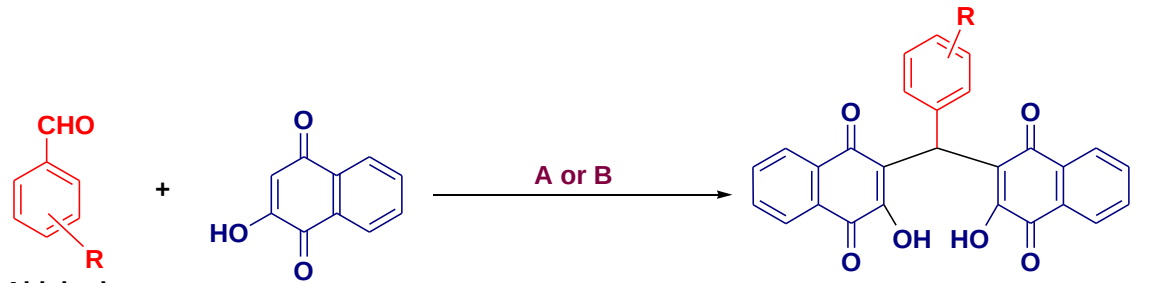
Apart from synthetic study we also study the effect of various agents like lemon, HCl , NaOH, NaOH , Acetic Acid, Ammonia ,Tea, Vicks, Clove oil, Mustard oil, Vinegar, Coffee Powder, Methi Seeds, Camphor, Clove solution on the λ_{max} of 2-Hydroxy-1,4-naphthoquinone, which is a principal dye in the leaves of henna. Also, the synthesized compounds were screened for antimicrobial activity.

5. **Research problem/hypothesis/objectives**

1. Green synthesis of henna based Scaffolds.
2. Study the antimicrobial activity of the synthesized compounds.
3. Study the variation in $\lambda_{max}$of Lawsone (2-Hydroxy-1,4-naphthoquinone) in different solution.

6. **Methodology Techniques/Sampling /Tools/Materials**

1. Green synthesis of henna based Scaffolds.



(A) Lemon juice (1mL), water: ethanol (1:1), 70^{o}C.
(B) Lemon juice (1mL), microwave irradiation.

2. Study the antimicrobial activity of the synthesized compounds

All Lawsone scaffolds were tested against *Escherichia coli* MTCC 82 strain.

The Disc Diffusion Method

Screening of the antibacterial activity of the test compounds was assessed by agar disc diffusion method according to recommendations of National Committee for Clinical Laboratory Standards [16]. Results were compared with that of lawsone. Stock solutions of the all the examined compounds (100 mg/ ml) were dissolved in DMSO. The cell density of the inoculum was adjusted with a UV-Visible spectrophotometer in order to obtain a final concentration of approximately 108 colony-forming units (optical density OD625 = 0.08-0.1). This suspension was used to inoculate on Mueller Hinton agar (MHA) by sterile cotton swab.

Whatman paper discs were loaded with different concentration of the test compounds and allowed to dry. These paper discs were placed on the inoculated MH agar plates. The anti microbial activity was determined by the measurement of the inhibitory zone diameter in mm after incubation at 37°C for 24 h. Ampicilin was used as antibacterial references. DMSO solvent was used as negative control. The antimicrobial activity was considered to start when the diameter was 6 mm or higher and was classified as follows: Very Strong activity: diameter ≥ 30 mm; Strong activity: diameter between 21-29 mm; Medium activity: diameter between 16-20 mm; Weak activity: diameter between 11-15 mm; Small or no activity: diameter ≤10 mm.

Macrodilution (Tube) Broth Method

Serial dilutions of test compounds: Prepared the serial dilutions using MH (Mueller Hinton) broth from the filter sterilized stalk of each of the test compounds. Transferred 1 ml of each of the dilutions to sterile glass tubes.

Preparation of inoculums: Prepared an inoculum for the agar dilution method by growing *E.coli* to the turbidity of the 0.5 McFarland standards, subsequently, diluted the suspension 1:150, resulting in a tube containing approximately 1×10^6 CFU/mL. Within 15 mins of inoculum dilution, added 1 ml of the adjusted inoculum to each tube containing 1 mL of antimicrobial agent in the dilution series (and a positive control tube containing only broth,), and mixed. This results in a 1:2 dilution of each antimicrobial concentration and a 1:2 dilution of the inoculum. Incubated the inoculated macrodilution tubes at 37°C and overnight in an incubator shaker(14-16).
Determination of antimicrobial activity: After overnight incubation, 1:100 dilution of each tube was prepared and inoculated on MH agar plate for CFU count. After overnight incubation counted the colonies and compared the no of colonies with the CFU of initial inoculum.

3. Study the variation in λ_{max} of Lawsone (2-Hydroxy-1,4-naphthoquinone) in different solution i.e. lemon, HCl , NaOH, NaOH , Acetic Acid, Ammonia ,Tea, Vicks, Clove oil, Mustard oil, Vinegar, Coffee Powder, Methi Seeds, Camphor, Clove solution.

7. **Result and Discussion**

1. Green synthesis of henna based Scaffolds

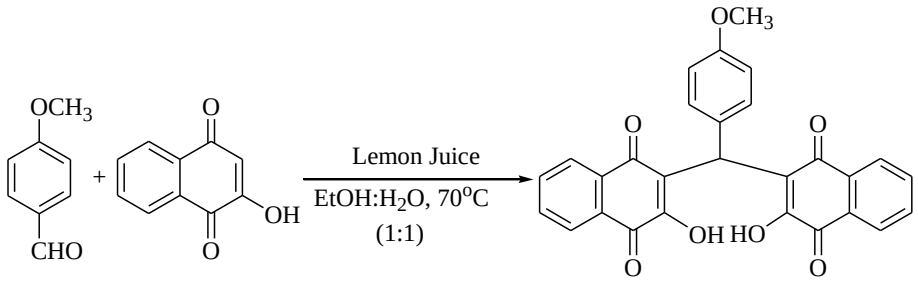
Aiming to achieve optimum reaction conditions for the synthesis of 3,3-arylmethylenebis(2-hydroxy naphthalene-1,4-diones), we investigated the condensation reaction of 4-methoxybenzaldehyde (**Ia**) (1.0 mmol) and 2-hydoxy-1,4-napthoquinone (**II**) (2.0 mmol) in different conditions. The reactions were carried out both at room temperature and at higher temperatures and in the presence of catalyst like HCl, Acetic acid, tomato juice, mosambi juice, coconut water and lemon juice. All the reactions were monitored by thin layer chromatography. The details of reactions carried out for optimization are listed in Table 1. It can be inferred from the table that, we get the best results by carrying out the condensation of **Ia** and **II** in the presence of lemon juice (1 mL) as a catalyst and water:ethanol (1:1) as a solvent at 70^{o}C. Increase in the amount of the lemon juice does not effects the yield or time of the reaction. Whereas decreasing the amount of catalyst increases the time of the reaction significantly.

Table 1: Effect of different media and catalyst on the condensation of 4-methoxybenzaldehyde (Ia) and 2-hydroxy-1,4-naphthoquinone (II)[a]

Run	Catalyst	Loading (mL)	Temperature	Reaction Media[d]	Time	(%) Yield (**IIIa)**
1.	None	-	70^{o}C	Water	24 h	-[b]
2.	None	-	70^{o}C	Ethanol	24 h	-[b]
2.	HCl	0.5	70^{o}C	Ethanol	5 h	-[c]
3.	Acetic acid	0.5	70^{o}C	ethanol	5 h	-[c]
4.	Tomato juice	1 mL	70^{o}C	Ethanol:water	50 min	85
5.	Mosambi juice	1 mL	70^{o}C	Ethanol:water	40 min	80
6.	Coconut water	1 mL	70^{o}C	Ethanol:water	70 min	86
7.	Lemon Juice	1 mL	70^{o}C	Ethanol:water	40 min	93
8.	Lemon Juice	2 mL	70^{o}C	Ethanol:water	40 min	93
9	Lemon Juice	0.5 mL	70^{o}C	Ethanol:water	70 min	88
10	Lemon Juice	0.5 mL	40^{o}C	Ethanol:water	3h	70

[a]Molar ratio, 4-methoxybenzaldehyde:2-hydroxy-1,4-naphthoquinone, 1:2.
[b]Incomplete reaction
[c]Sluggish and incomplete reactions.
[d]Solvent (10 mL)

From the above observations, it is clear that lemon juice efficiently catalyzes the condensation of 4-methoxybenzaldehyde and naphthoquinone to afford 3,3-(4-methoxy phenyl)methylenebis(2-hydroxynaphthalene-1,4-dione) (IIIa) in ethanol:water (1:1, v/v).



3,3-(4-methoxyphenyl)methylenebis(2-hydroxynaphthalene-1,4-dione)

The optimized protocol was extended to other aromatic aldehydes such as benzaldehdye (Ib), 4-nitrobenzaldehyde (Ic), 4-chlorobenzaldehyde (Id). All these aldehydes underwent successful condensation with 2-hydroxy-1,4-naphthoquinone using 1 mL of lemon juice in EtOH:H_2O (1:1, v/v) at 70^oC to afford the corresponding 3,3-(4-phenyl)methylenebis(2-hydroxy naphthalene-1,4-dione) (IIIb), 3,3-(4-nitrophenyl)methylenebis(2-hydroxynaphthalene-1,4-dione) (IIIc), 3,3-(4-chlorophenyl) methylenebis(2-hydroxynaphthalene-1,4-dione) (IIId) in high yields. These results are compiled in Table 2.

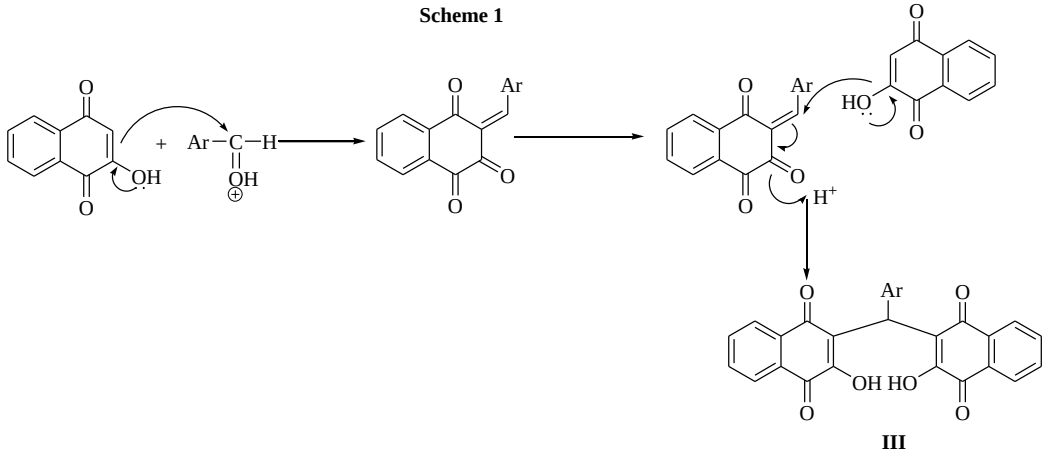
Table 2: Synthesis of 3,3-arylmethylenebis(2-hydroxynaphthalene-1,4-dione) (III) using Lemon juice as catalyst[a]

Run	Ar	Product	Time (min)	(%)Yield**(II)**
11.	4-$CH_3OC_6H_4$ (Ia)	**IIIa**	40	93
12.	C_6H_5 (Ib)	**IIIb**	45	90
13.	4-$NO_2C_6H_4$ (Ic)	**IIIc**	35	89
14.	4-ClC_6H_4 (Ie)	**IIId**	45	92

[a]4-methoxybenzaldehyde:2-hydroxy-1,4-naphthoquinone (1:2molar ratio).

A probable mechanism for the formation of 3,3-arylmethylenebis(2-hydroxynaphthalene-1,4-dione) from the condensation of aromatic aldehyde and 2-hydoxy-1,4-napthoquinone is given in Scheme 1.

Scheme 1



III

Further, the effect of the microwave irradiation was also investigated on this condensation reaction. The reaction of 2-hydroxy-1,4-napthoquinone (**II**) and 2-methoxybenzaldehyde (**Ia**) was attempted at 70^oC at 150 W power and 1 mL of lemon juice. The reaction was found to complete after 7 min with a yield of 93% of **IIIa**.Reaction at higher temperature (150^oC) did

not affect the yield or reaction time whereas reaction at lower of temperature (40^{o}C) affects the yield of **IIIa** significantly. Similar results were obtained on changing the power from 150 W to 200 W and 50 W. It can be concluded from the results that 1mLof lemon juiceunder microwave irradiation (150 W, 70^{o}C) catalyzes the condensation reaction 2-hydroxy-1,4-napthoquinone (II) and 2-methoxybenzaldehyde (Ia) efficiently to afford the corresponding xanthene derivative **IIIa** in high yield. Similarly other compounds were synthesized under similar condition.

2. Antimicrobial activity of the synthesized compounds

Disc Diffusion method

Unfortunately all the scaffolds had very poor solubility in water and DMSO, whereas Lawsone dissolved very well in the DMSO. Because of the poor solubility the test compounds could not diffuse out of the paper discs used for disc diffusion method. Various solvents were tried but the diffusion of the compounds could not be achieved, thus disc diffusion method could not show any zone of inhibition on E. coli MH agar plate. The scaffolds could be dissolved in acetone, but acetone has its own antimicrobial property, therefore acetone was also not a good solvent to be used for disc diffusion method.

Macro dilution method:

An alternate broth dilution method for studying antimicrobial property was used. Dilutions of scaffolds/test compounds made in MH broth gave very turbid solutions, therefore the antimicrobial activity could only be determined by CFU(Colony Forming Unit) method instead of the conventionally used O.D. method (Optical Density).

The OD of the culture was adjusted to 0.08= 1.5-2x10^{8} CFU/ml.

Dilution of the culture 1:100=10^{7} CFU /ml.

The volume of the *E. coli* culture added to the sterile glass tube=1ml

The dilution of test compound added=1ml

The final CFU of the inoculums in each tube after addition of test compound=5x10^{6}CFU /ml(To)

CFU= No of colonies X Dilution Factor

Dilution Factor = Total volume of the CFU assay tube X dilution of the assay X volume plated (2000)

The reduction in CFU calculated using formula:

(CFU of inoculums at To-CFU of culture in the presence of compound after overnight incubation)X100

CFU of inoculums at To

Table 3: reduction in CFU in the presence of the parent compound lawsone and all four scaffolds show the antimicrobial property. The scaffolds are: 4-$CH_3OC_6H_4$ (Ia), C_6H_5 (Ib), 4-$NO_2C_6H_4$ (Ic) and 4-ClC_6H_4 (Ie).

S.No	Compound	Concentration Of the compound (mg/ml)	CFU/ml	%Reduction in CFU
1	Lawsone	0.5	Lawn of colonies	No significant reduction
		1.25	$2x10^6$	20%
		2.5	$3.04\ x10^6$	40%
		5	$1.8\ x10^6$	64%
		10	$9.6\ x10^5$	84%
		20	No colonies	100%
2.	4-$CH_3OC_6H_4$	1	lawn	No significant reduction
		2.5	lawn	No significant reduction
		5	$3.7\ x10^6$	26%
		10	$2.1\ x10^6$	58%
		20	$1\ x10^6$	80%
		40	No colonies	100%
3.	4-ClC_6H_4	1	lawn	No significant reduction
		2.5	$4.4\ x10^6$	12%
		5	$3.5\ x10^6$	30%
		10	$2.4\ x10^6$	50%
		20	$9\ x10^5$	82%
		40	No colonies	100%
4	$NO_2C_6H_4$	1	lawn	No significant reduction
		2.5	lawn	No significant reduction
		5	$3.6\ x10^6$	28%

		10	2.4×10^6	50%
		20	8.1×10^5	84%
		40	No colonies	100%
5.	C_6H_5	1	lawn	No significant reduction
		2.5	lawn	No significant reduction
		5	3.3×10^6	36%
		10	2.3×10^6	54%
		20	1×10^6	80%
		40	No colonies	100%

The compounds showed antimicrobial activity at high concentration which can be due to their poor solubility in the media. This can be further resolved with more work on finding better and efficient solvent. But these preliminary studies show the potential of these compounds as antimicrobial compounds.

3. Spectrophotometrically evaluation of λ_{max} of 2-Hydroxy-1,4-naphthoquinone in different solution.

The electronic absorption spectra of 2-hydroxy-1,4-napthoquinone (0.1% in water) was measured in different solutions like lemon juice , HCl , NaOH, NaOH , Acetic Acid, Ammonia ,Tea, Vicks, Clove oil, Mustard oil, Vinegar, Coffee Powder, Methi Seeds, Camphor, Clove solution and the corresponding values of λ_{max} are summarized in Table 4.

Table 4: The electronic absorption spectra of 2-hydroxy-1,4-napthoquinone in different solutions.

S.NO.	Component (Deionized water based solutions)	λ_{max}
1.	Napthoquinone	423
2.	Lemon Juice	380
3.	Conc. HCl	380
4.	Dil. HCl	381
5.	Conc. NaOH	430
6.	Dil. NaOH	427
7.	Acetic Acid	388
8.	Ammonia	426
9.	Tea	418
10.	Vicks	416
11.	Vicks Overnight	440
12.	Clove Oil	439
13.	Mustard Oil	435
14.	Vinegar	439
15.	Coffee	388
16.	Methi seeds	492

17.	Camphor	431
18.	Clove Solution	429

From the spectrophotometric studies, we have analyzed that the addition of methi seeds solution have an marked effect on the λ_{max} valueof 2-hydroxy-1,4-naphthoquinone.The addition of acid solution such as dilHCl, lemon, glacial acetic acid decreases the λ_{max} value of 2-hydroxy-1,4-naphthoquinone.The addition of base (NaOH), tea, vicks, clove oil, coffee etc. does not have any marked effect on the λ_{max} value.

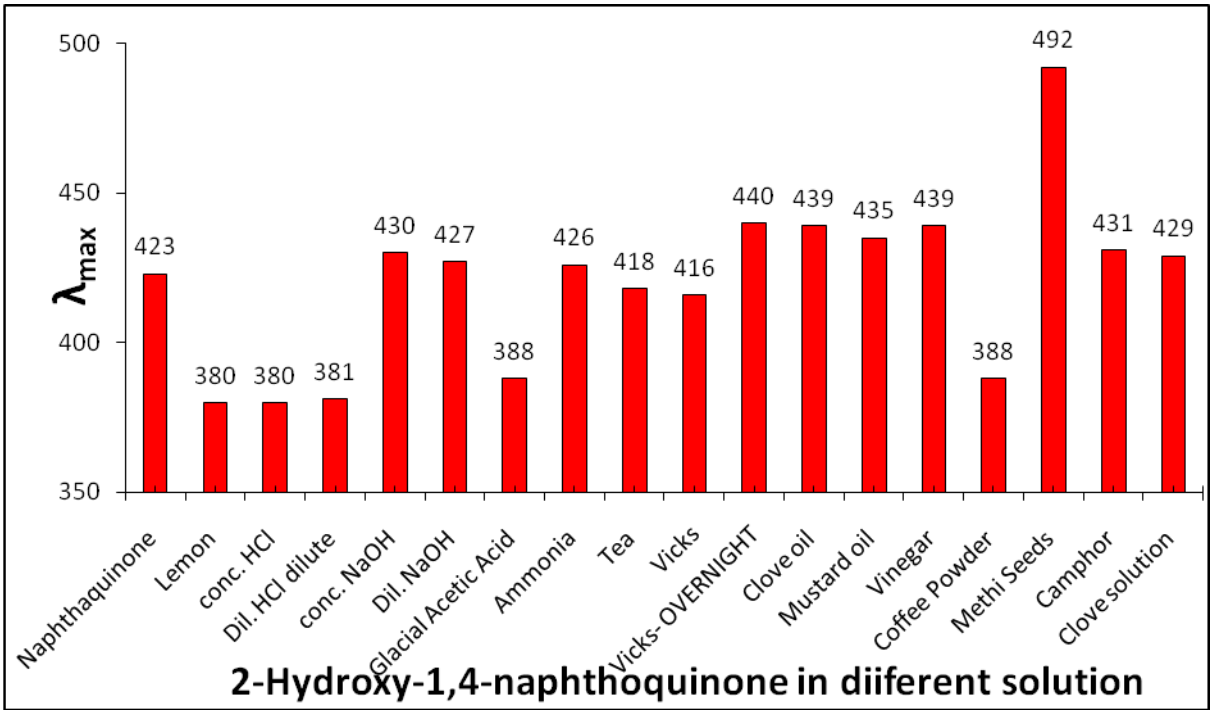


Figure 1.λ_{max}values of 2-hydoxy-1,4-naphthoquinone in different solutions

8. Innovations shown by the project

1. We have scientifically developed a novel and green methodology for the synthesis of 3,3-arylmethylenebis(2-hydroxynaphthalene-1,4-dione) compounds by using simple, cheap and easily available catalyst.

2. We have scientifically related the color and λ_{max} value of 2-hydroxy-1,4-naphthoquinone which is a color imparting component of henna. We have given in vitro effect of number of solution on the color of the henna.

3. We have observed the antimicrobial activity of the all four, 3,3-arylmethylenebis(2-hydroxynaphthalene-1,4-dione) compounds, which were comparable to parent compound's

known antibacterial properties. This can prove to be beneficial for use in pharmaceutical industry.

9. Conclusion and Future direction

1. In conclusion, we have devised efficient synthetic approachesfor the synthesis of 3,3-arylmethylenebis(2-hydroxynaphthalene-1,4-dione) derivatives. The condensation has been proficiently performed in water as a"green" solvent using lemon juiceas catalyst. The advantages of thismethod include operationalsimplicity, high yields, and easy availability of the catalyst.

2. From the spectrophotometric studies, we have analyzed that the addition of methi seeds solution have an marked effect on the λ_{max} value of 2-hydroxy-1,4-naphthoquinone.The addition of acid solution such as dilHCl, lemon, glacial acetic acid decreases the λ_{max} value of 2-hydroxy-1,4-naphthoquinone. The addition of base (NaOH), tea, vicks, clove oil, coffee etc. does not have any marked effect on the λ_{max} value.

3. The high concentrations at which these compounds showed the antibacterial activity could be an attribute of impurities present in the compounds and the poor solubility of the scaffolds.More work on finding the correct solvent and further antimicrobial studies with different bacterial strains and fungi can be useful in adding newer, more green and eco friendly antimicrobial compounds for pharmaceutical industry.

Henna is a part of many Indian traditions and extensively used in all the festivals, so the scientific findings can be used in future to develop a herbal product for better coloring properties of henna.

3.

10. References in APA format

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12. Conference Presentation/s (attach copies)

1. Presented the poster "Green Protocol for the Synthesis of Henna (Lawsonia inermis) based Scaffolds using lemon Juice" in RSC workshop on "Green chemistry and Water Treatment" on 17th October 2016, New Delhi.

2. Presented the poster "Variation on λ_{max} value of Lawsone (2-hydroxy-1,4-napthoquinone) in different solution: A preliminary study" in "National Conference in Chemistry

Environment and Harmonious Development" organised by Shyam Lal College, Delhi, 7-8 April 2016.

15. Pictures related to the project.

Reaction in Synthetic Microwave

Reaction under reflux mounted over the magnetic stirrer