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Microwave Assisted Rapid Extraction and Characterization of Coumarin from Fig Plant (*Ficus carica*)

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***Abstract* - Coumarins are fragrant natural bioactive organic compound find application as aroma, flavor and wide medicinal use. Fig plant (*Ficus Carica*) is a very good source of Coumarins. Coumarins are a member of the benzopyrone family and it has a benzene ring joined to a pyrone ring. There is a need for effective extraction and purification of Coumarins from plant materials. Different methods of extraction of Courmarin from *Ficus Carica* has been studied in this paper, among them, microwave extraction (180 W power) provided rapid extraction with 10 minutes time with extract yield of 0.67 g as compared to that of 1 hour of water bath extraction at 60°C. Coumarin was isolated from the crude extract using NaOH and petroleum ether and their presence was characterized confirmed using UV spectroscopy, IR spectroscopy, TLC and fluorescence test. The antibacterial activity of coumarin was also evaluated using disc diffusion method and Minimum Inhibition Count (MIC). The present study analyzes and provides method of microwave assisted rapid extraction of Coumarin from Fig plant (*Ficus Carica*) and its characterization, which could be widely used as generic method from natural materials for different applications.**

***Keywords:* Fig plant (Ficus Carica), Coumarin, Extraction Method, Microwave, Antibacterial**

I. INTRODUCTION

Coumarins are colourless, crystalline polyphenolic compounds having wide range of pharmaceutical actions, biological functions and also used for treating various clinical conditions [1]. They are oxygen heterocycle and belong to the family of benzopyrones, which consists of benzene ring joined by a pyrone ring [2, 3]. Although distributed throughout all parts of the plant, the coumarins occur at the highest levels in the fruits, followed by the roots, stems and leaves [4]. There are different types of coumarin. Coumarins are categorized into four main types are simple coumarins (e.g. Coumarin), furanocoumarins (e.g. Psoralen), pyranocoumarins (e.g. Xanthylein) and pyrone substituted coumarins (e.g. Warfarin) [5]. Simple coumarins consists of hydrolylated, alkoxylated or alkylated benzene ring whereas furanocoumarins and pyranocoumarins have furan ring and pyran ring attached to benzene ring and pyrone substituted coumarins have substitution on pyrone ring. Coumarins (Figure 1) are synthesized as secondary metabolites and distributed across different parts of the plants, and also they have specific histological locations in the tissues. Within the plant they are most abundant in fruits and roots. However, in flowers and leaves they are evident in fewer quantities. In some plant species Coumarins were also found in the bark or stems [6].

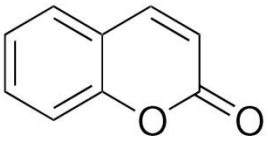

Fig.1 Structure of Coumarin

Coumarins are distributed across different parts of the plants, and they have specific histological locations in the tissues. Within the plant they are most abundant in fruits and roots. However, in flowers and leaves they are evident in fewer quantities. In some plant species coumarins were also found in the bark or stems [7]. Some important coumarin members have been isolated from microbial sources e.g. novobiocin and coumermycin from *Streptomyces*, and aflatoxins from *Aspergillus* species [8, 9].

F. Carica is an important member of the genus Ficus. It is ordinarily deciduous and commonly referred to as "fig". Fig (*Ficuscarica*, *Moraceae*) products are widely used both as food and as medicine in the Middle East [10].The dried fruits of *F. Carica* have been reported as an important source of vitamins, minerals, carbohydrates, sugars, organic acids, and phenolic compounds. The fresh and dried figs also contain high amounts of fiber and polyphenols. Figs are an excellent source of phenolic compounds, such as proanthocyanidins, whereas red wine and tea, which are two good sources of phenolic compounds, contain phenols lower than those in fig. Its fruit, root, and leaves are used in traditional medicine to treat various ailments such as gastrointestinal (colic, indigestion, loss of appetite, and diarrhea), respiratory (sore throats, coughs, and bronchial problems), and cardiovascular disorders and as anti-inflammatory and antispasmodic remedy. Phytochemical studies on *F. Carica* revealed the presence of numerous bioactive compounds such as phenolic compounds, phytosterols, organic acids, anthocyanin composition, triterpenoids, coumarins, and volatile compounds such as hydrocarbons, aliphatic alcohols, and few other classes of secondary metabolites from different parts of *F. carica*. There is a need for effective extraction and purification of Coumarins from plant materials. Different methods of extraction such as use of Microwave, Water bath heating, Magnetic stirring and characterization of Courmarin from *F. Carica* has been studied in this paper.

II. EXPERIMENTAL STUDY

A. Materials

Fig fruits were collected from the market and sun-dried. Then they were made into powder using a domestic mixer. Lab grade reagents such as Lead acetate, Chloroform, Ethyl acetate, Acetic acid and Petroleum ether was purchased from Merck specialties Pvt. Ltd. Hexane, Acetone and Sodium hydroxide was purchased from S D Fine Chem limited. Methanol and Silica gel (60-120 mesh) was purchased from Sigma Aldrich and Himedia laboratories Pvt. Ltd.

B. Methods

1. Extraction of Courmarin: For the extraction of crude extract from the fig fruit powder, three methods were used such as microwave, water bath and magnetic stirrer extraction.

2. Microwave Assisted Extraction: Samsung Microwave oven with 28 L capacity generating Microwave at 2.45 Ghz was used in the extraction experiment. 5 g of Fig fruit powder was dissolved in 50 ml distilled water. Then this solution was transferred to a microwave dish. The experiment was carried out at various temperature and time and extract was collected (2 ml and 5 ml). After the extraction the solution is allowed to cool at room temperature and filtered using Whatman filter paper.

3. Water Bath Extraction: 5 g of powdered fruit were dissolved in 50 ml methanol. This solution was kept in water bath at 60˚C for 2 hours. The sample was collected at every 1 h of treatment (2 ml and 5 ml). Then the extract filtered using Whatman filter paper.

4. Magnetic Stirrer Extraction: 5 g of fruit powder was dissolved in 50 ml methanol and kept in magnetic stirrer for 5 hours. The sample was collected at every 1 h of treatment and the extract is filtered using Whatman filter paper.

C. Isolation of Courmarin

1. Removal of Sugar and Tanning Agent: 1 g of Lead acetate was mixed with the above crude extract and the sugar and tannins present in the extract got precipitated after few minutes. After filtering the sample filtrate was collected and stored.

2. Isolation of Coumarin Using Column Chromatography: The column was packed uniformly using 25 g of silica gel (60-120 mesh) which is dissolved in 100ml of chloroform. Then the column was run with chloroform to avoid improper packing. 3 g of silica gel were dissolved in the above sugar and tannin free extract. This sample was added on the top of the column and allowed for absorption. After absorption ethyl acetate was added in the column followed by hexane.The samples got separated in the form of layers in the column. Coumarin got separated as yellow color band and chlorophyll got separated as green color band.Using acetone coumarin and chlorophyll was collected in separate beaker and stored.

3. Isolation Using Petroleum Ether: 20 ml of petroleum ether was added to the sugar and tannin free extracts and kept in orbital shaker at 600 rpm for 10 minutes. The Coumarin will get separated in the organic phase since it is insoluble in petroleum ether. Then the organic phase was allowed to condense and extracted it with methanol. Then again the extract was allowed to condense and recrystallized using ethanol.

4. Isolation Using Sodium Hydroxide (Structural Based Isolation (Lactone Type): 20 ml of 0.1 N NaOH was added to the fig fruit powder (5 g) and filtered the solution. The NaOH will break the lactone ring and release coumaric acid. Acetic acid was added to the solution, this will cyclize the coumaric acid to coumarin. Then using ethanol condensed it and recrystallized the isolate.

D. Characterization and Chemical Confirmation Tests

1. Characterization:

a. UV-Visible Spectroscopy: This characterization was done using spectrum method. 0.05 mg of extract was dissolved in methanol and made upto 10 ml. Methanol was used as reference. The spectrum was used in the range of 200-700 nm.

b. IR Spectroscopy: IR Spectroscopy was used to analyze the functional group present in the extract and its stretching frequency. The sample is mixed with a pinch of KBr in a mortar and pestle and made into a disk (KBr pellet). The pellet was placed in the sample holder and the transmittance was seen.

c. Thin Layer Chromatography: Chromatography is used to separate mixtures of substances into their components TLC was used to investigate whether the compound is isolated or not. The R_f value for each sample is worked out using the formula:

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

Silica gel was used as an absorbent and mobile phase was varied as following:

1. Ethyl acetate, acetone, formic acid, water (5:3:1:1)
2. Hexane, ethyl acetate, formic acid (5:4:1)
3. Hexane and chloroform (8:2)

2. Chemical Conformation Test: 1 mg of extract was dissolved in 2 ml of methanol and 2 drops of 0.1 N NaOH was also added to this. If this sample emits yellow fluorescence under UV light, it confirms the presence of coumarin.

a. Antibacterial Activity Test: This test was carried out using disc diffusion method. For this 1 mg of soil sample was collected near tannery division of CSIR- CLRI. Serial

dilution was prepared up to 10^{-6} dilution. Then the nutrient agar plates prepared and inoculated with 10^{-6} dilution using spread plate technique. Then 12 discs containing minimum amount of sample is placed over the inoculated plates and incubated for 48 h.

b. Minimum Inhibition Count (MIC) Test: Minimum inhibition count is carried out to find the minimum inhibition zone. Microorganisms can be tested for their ability to produce visible growth on a series of agar plates (agar dilution), in tubes with broth (broth dilution), or in microplate wells of broth (broth micro dilution) containing dilutions of an antimicrobial agent. MIC is defined as the lowest antibiotic concentration that prevents visible growth of bacteria. MIC methods are widely used in the comparative testing of new agents, or when a more accurate result is required. Sterile capped test tubes 9 nos. were taken and numbered as 1 to 9. All of the following steps are carried out using aseptic technique. Then 2 ml of isolated Coumarin sample solution (100 µg/ml) was added to the first tube and 1 ml of sterile broth was added to all other tubes. Transfer 1.0 ml of sample from the first tube to the second tube. Then mixed the contents of this tube using separate pipette and transfer 1 ml from this to the third tube. Continued the dilutions in this manner to tube number 8, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette. Removed 1 ml from tube 8 and discard it. The ninth tube, which serves as a control, received no sample. Suspend to an appropriate turbidity several colonies of the culture to be tested in 5.0 ml of Mueller-Hinton broth to give a slightly turbid suspension. This suspension was diluted by aseptically pipetting 0.2 ml of the suspension into 40 ml of Mueller-Hinton broth. 1 ml of the diluted culture suspension was added to each of the tubes. The final concentration of tetracycline is now one-half of the original concentration in each tube. Incubate all tubes at 35°C overnight. Then tubes were examined for visible signs of bacterial growth after 24 hours. The highest dilution without growth is the Minimal Inhibitory Concentration (MIC).

III. RESULTS AND DISCUSSION

A. Extraction of Coumarin

TABLE I RESULTS OF MICROWAVE EXTRACTION WITH VARYING POWER AND TIME

Power (W)	Time (min)	Weight of extract (g)
100	25	0.0248
180	10	0.6720
350	5	0.0520
450	2.50	0.0314
600	2	0.0241
900	1.50	0.0423

Among three extractions used, water bath and microwave extraction gave the best result. The results of extraction using microwave (with varying power and time) and water bath are given in Table I and II. The results indicate that amongst different methods of extraction of Coumarin from *F. Carica*, microwave extraction (180 W power) provided rapid extraction with 10 minutes time with extract yield of 0.67 g as compared to 1 hour of water bath extraction at 60°C.

TABLE II RESULTS OF WATER BATH EXTRACTION WITH VARYING TIME

Hours	Weight of the extract (g)
1	0.67045
2	1.0181

B. Isolation of Coumarin

Isolation using NaOH and petroleum ether gave the best results. For 5 g of fruit powder NaOH extraction gave about 3.4321 g yield and petroleum ether gave 2.6821 g.

C. Characterization and Chemical Confirmation Tests

1. Characterization

a. UV-Visible Spectroscopy: The characterization results of NaOH extract and ether extract was given in Table III and IV. The UV visible ranges from 274nm and 311nm and fluorescence range 331nm and 214nm shows presence of substituted Coumarin.

TABLE III UV ANALYSIS RESULTS OF NAOH EXTRACT

Wavelength	Absorption
331	0.021
241	3.965

TABLE IV UV ANALYSIS RESULTS OF ETHER EXTRACT

Wavelength	Absorption
310	0.692
274	1.152
210	2.225

b. IR Spectroscopy: The stretching frequency in the range of 1700-1750cm^{-1} shows the presence of C=O in the extract, skeletal vibrations in the range of 1600-1660 cm^{-1} shows the presence of C=C in the extract and stretching frequency 1500cm^{-i} indicate the presence of aromatic ring. Figure 2 shows the FT-IR spectrum of the extract from the fruit.

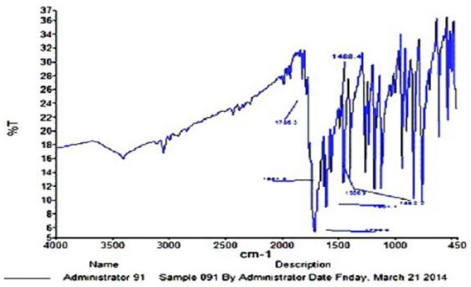

Fig. 2 IR spectrum of fruit extract

c. Thin Layer Chromatography: TLC conform the isolation and presence of Coumarin and its R_f value is 0.2567.

2. Chemical Conformation Test

a. *Fluorescence Test:* Confirm the presence of coumarin in the fruit extract. Fig.3 shows the image of fruit extract emits yellow fluorescence under UV light.

Fig. 3 Image of fruit extract fluorescence under UV light

b. Antibacterial Activity Test: Disc diffusion test shows that Coumarin has good antibacterial activity. Figure 4shows the image of antibacterial activity of coumarin using disc diffusion method. Microorganisms have shown a visible growth on a series of agar plates from 10^{-1} to 10^{-6} dilution, in tubes with broth containing dilutions of Coumarin as antimicrobial agent. The Figure 5 shows MIC of 10^{-1} -10^{-2} dilution of 100 µg/ml of Coumarin works out to be effective in inhibition of bacterial growth.

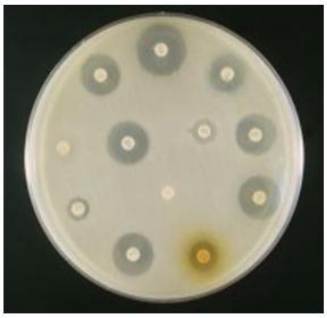

Fig. 4 Antibacterial activity of Coumarin isolated from

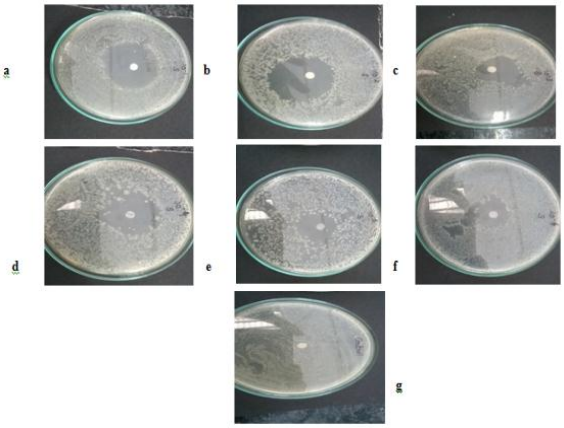

Fig. 5 Minimum inhibition count (MIC) of different dilutions of sample
a) 10^{-1} b) 10^{-2} c) 10^{-3} d) 10^{-4} e) 10^{-5} f) 10^{-6} g) Control

IV. CONCLUSION

One of the bioactive compounds present in the *Ficus Carica* is Coumarin and found to be more in the fruit than the leaves. Different methods of extraction of Courmarin from *Ficus Carica* has been studied, among them, microwave extraction (180 W power) provided rapid extraction with 10 minutes time with extract yield of 0.67 g as compared to that of 1 hours of water bath extraction at 60°C. From the above studies it was found that, structural based isolation (using NaOH) and petroleum ether isolation could be used as an isolation method for Coumarin from the crude extract. Among these two methods, structural based isolation is the best method for isolation of coumarin as it gives more yield compare to other method. The analysis of the sample under UV-Visible spectroscopy, FT-IR and TLC conformation test shows that, UV visible rangesfrom 274nm and 311nm and fluorescence range 331nm and 214nm shows presence of unsubstituted coumarin. And FT-IR results indicates presence of C=O, C=C and aromatic ring. TLC analysis gave R_f value of Coumarin isolated from crude extract is 0.2567. Fluorescence test again confirmed the presence of Coumarin in the extract. Finally, the antibacterial test using disc method proved that the Coumarin is bacteriostatic. Hence, the present study provides method of microwave assisted rapid extraction of Coumarin from Fig plant (*Ficus Carica*) and its characterization, which could be widely used as generic method from natural materials for wide applications.

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