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Phytochemical and bioactive compounds identification of *Ficus auriculata* fig methanolic extraction and its antioxidant activity

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Abstract

In the current investigation, a new phytoconstituent was isolated and identified from a methanolic extract of *Ficus auriculata* Lour figs. Naturally occurring phytoconstituents have been used to treat ailments for a long time and have served as a source of structural variety for natural product chemists. Natural products are frequently mentioned as a rich source of chemical variation when looking for new pharmacological leads or molecules. The analysis of the chemical components of the methanolic extract led to the discovery of a new phytoconstituent, 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one, with pyran as a basic nucleus. *Ficus auriculata* Lour figs were collected and sun-dried. Dried *Ficus auriculata* Lour figs were extracted using menthol. *Ficus auriculata* Lour figs methanolic extract conducted a preliminary phytochemical study. *Ficus auriculata* Lour figs methanolic extract novel phytoconstituent isolated and identified by using FT-IR, 1HNMR, 13CNMR, and MS were used to determine the compound's structure. Methanolic extract conducted antioxidant activity by the DPPH method. Preliminary screening confirmed that methanolic extract contains flavonoids and phenols. The purity of the 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one compound was confirmed by RP-HPLC. 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b]pyran-4-one compound were novel and first reported in *Ficus auriculata* Lour methanolic extract in the Ficus family. Based on previous studies, pyran moieties have a significant role in biological activities. Methanolic extract showed good antioxidant activity as compared to ascorbic acid. From the methanolic extract of the *Ficus auriculata* Lour fig, the chemical A, 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one, was successfully isolated and identified. FT-IR, 1HNMR, 13CNMR, and LC-MS/MS spectral data were used to determine the novel compound's structure. Methanolic extract of *Ficus auriculata* Lour fig showing good antioxidant activity.

Others: The isolated new pyran derivative might be helpful in creating lead moieties that can be used in various ways.

Keywords: *Ficus auriculata*; Methanolic extract; DPPH assay; Phytoconstituent; LC-MS; NMR; TLC

1. Introduction

Billions of people in need of traditional medications based on plants live in nations that are still developing. This number shows the percentage of the world's population for which conventional medicine is the primary means of accessing healthcare services. According to the definition provided by the World Health Organization, a medicinal herb is any plant that has compounds in one or more of its organs that have the potential to be utilised for therapy [1]. They are also the source of semi-synthesis in chemo-pharmaceutical compounds. Because the grains or seeds, fruits, flowers, barks, stems, rhizomes, roots, and leaves of plants in this genus contain chemical components that are active in medicine, they are utilised in the prevention and treatment of disease. In addition, these bioactive components of

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chemical substances found in plants are typically referred to as phytochemicals [1]. They also play a crucial role in defending plants from pest-induced microbial infestations or illnesses.

Meanwhile, phytochemistry is regarded as the research on natural products [2]. Phytochemicals have been isolated and described from various food and drink sources, including spices like turmeric, beverages like green tea and red wine, leafy green vegetables and onion, fruits like grapes and apples, and several other sources. Ethnopharmacology refers to the branch of pharmacological study that emphasises the utilisation of traditional or regional medical practises, such as using plants for the treatment of illnesses. That behaviour, however, has been around since prehistoric times. In addition, ethnopharmacology has only relatively recently been integrated into contemporary medical practice, even though it is a significant traditional medicine used worldwide [3]. The use of conventional medicine dates back centuries in India. The Indian *Materia Medica* provides a wealth of information on the folkloric practises and traditional use of naturally occurring substances that have potential medical applications [4, 5].

Although many plants are utilised in traditional medicine, there is no scientific investigation of many plants. Medicinal plants have a long history of being used as a source of many active ingredients used to treat human ailments and are rich in therapeutic value [6]. More than 800 species and 2000 variants of the Ficus genus can be found across the world's tropical and subtropical woods, making it one of the largest genera of angiosperms [7]. The most popular species in the genus, known as fig trees, is the common fig (*Ficus auriculata*), which yields the commercial fruit known as fig [8]. The current investigation aims to identify the phytochemical and bioactive components in methanolic-extracted Ficus auriculata figs and their antioxidant activity.

2. Material and methods

2.1 Extraction

After the collection, figs of *Ficus auriculata* were carefully washed with deionised water to eliminate undesirable materials, and cleaned figs were sun-dried for two weeks. They milled with a suitable mill into a fine powder. 500 g of fine powder was soaked in 1000 ml of methanol and kept at room temperature for two weeks. After two weeks, they filtered with the Whatman filter paper and concentrated using a rotary evaporator. It formed a black sticky concentrate. The back-sticky concentrate was elected as a crude methanol extract [9].

2.2 Qualitative Estimation of Phytochemicals

The presence of the preliminary phytochemical was evaluated using the following conventional techniques for medicinal plants and methanolic and ethanolic aqueous solutions [10].

Flavonoids: 2 ml of the extract was treated with 2 ml of 10% lead acetate solution. The appearance of yellowish-green colour indicates the presence of flavonoid.

Alkaloids: 2 mL of extract and 2 mL of Wagner's reagent are added. A brownish precipitate indicates the presence of an alkaloid.

Phenols: 10 mg of plant extract was mixed with 0.5 ml of Lead acetate (10%) and observed for the presence of a white residue.

Tannins: 2 mL of each plant extract was mixed with a few drops of 0.1% $FeCl_3$ solution in test tubes. Test tubes were observed for the appearance of a brownish-green colour.

2.3 Quantitative Estimation of Phytochemicals

Various extracts of *Ficu ariculata* fig quantitative estimation of phytochemical analysis was evaluated using the spectroscopic method [11].

2.4 Estimation of Flavonoids

The total flavonoid concentration was calculated using the aluminium chloride colourimetric test. The reaction mixture of 1 mg S of extract and 4 ml of distilled water was placed in a 10 ml volumetric flask. 0.3 ml of mixed 10% aluminium chloride was added to the flask after 0.30 ml of treated 5% sodium nitrite. Before being made into 10 ml by diluting 2 ml of 1M sodium hydroxide with distilled water, the solution was treated for 5 minutes. A series of reference standard solutions of morin (20, 40, 60, 80, and 100 g/ml) were created in the same manner as previously mentioned. The

absorbance of the test and standard solutions at 510 nm was measured using a UV/visible spectrophotometer compared to the reagent blank. The total flavonoid concentration was determined as mg of ME/g of extract.

2.5 Estimation of Phenol

Extract required volume in each tube was brought up to 3.0 ml using pure water, and the total volume was recorded. After adding the Folin-Ciocalteau reagent (0.5 ml), the tubes were heated in a boiling water bath for exactly one minute. After cooling the tubes, an absorbance reading was taken at 650 nm in a spectrophotometer using a reagent blank as a reference. Standard gallic acid solutions ranging from 0.2 to 1 millilitre with concentrations ranging from 2.0 to 10 micrograms were also handled in the same manner as described above [12, 13].

2.6 Estimation of alkaloids

The plant extract (1 mg) was dissolved in dimethyl sulphoxide (DMSO), and 1 ml of 2 N HCl was added before filtration. After being transferred to a separating funnel, this solution was mixed with 5 ml phosphate buffer and 5 ml bromocresol green solution. Before being collected in a 10-ml volumetric flask and chloroform-diluting to the necessary volume, the mixture was rapidly agitated with 1-, 2-, 3-, and 4-ml chloroform. A series of atropine reference standard solutions (20, 40, 60, 80, and 100 g/ml) were created in the same manner as previously mentioned. The absorbance of the test and standard solutions was measured at 470 nm using a UV/Visible spectrophotometer in contrast to the reagent blank. The total alkaloid content was represented as mg of AE/g of extract (14).

2.7 Isolation and Identification of phytochemicals

The structure of molecules is ascertained using information from various spectroscopic techniques, such as UV-visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and mass spectroscopy. The essential concept behind spectroscopy is that electromagnetic energy is passed through a molecule, which absorbs some of the radiation but not all of it. A spectrum can be produced by calculating the amount of electromagnetic radiation drank. A molecule's spectra are specific to certain bonds. These spectra can be used to determine the organic molecule's structure. Researchers generally use spectra generated from three or four regions for structural confirmation. Electron beam, radio frequency, visible, infrared (IR), and ultraviolet (UV) light.

2.8 Isolation of phytocompounds

2.8.1 Column Chromatography

The methanolic extract of this Ficus was subjected to silica gel chromatography using a methanol: acetonitrile (3: 2) ratio. The eluting solvent was collected and concentrated using vacuum evaporator. The purity of the final single active compound was confirmed by TLC methanol: acetonitrile: formic acid (80:20:0.1% (v/v).) with 0. 52 Rf value. The final eluent was recrystallized by Ethanol (Room Temperature). The melting point of this compound is 71-73°C.

2.8.2 Compound characterization

The biologically active Ficus extract Fraction 1 (F1) was used for its purity analysis. The purity of F1 was analysed by reversed-phase high-performance liquid chromatography (RP-HPLC- Shimadzu LC-10 system, Shimadzu Co., Kyoto, Japan) coupled with PDA detector by Zhang et al. 2015. The C_{18} column was used for separation (100×4.60mm 2.6 µm, 100 Å) and the column temperature was maintained at 35°C. The gradient elution was used for HPLC analysis. A-water+0.1% formic acid, B-methanol:acetonitrile:formic acid (80:20:0.1% (v/v)). The gradient elution started at 90:10 (A: B) and shifted to 10:90 (A: B) for 18 min. 10µl injection volume and 280 nm wavelength were employed.

2.8.3 Fourier-transformed infrared (FTIR) spectra

The functional group present in F1 was analysed using FTIR analysis. KBr powder was combined with the optimized extract to produce 1% (w / v) slurry concentration, and the KBr pellet was prepared by pressing approximately 5.5 tons for 3 min. The measurements were then performed with a resolution of 4 cm−1 on a JASCO FT / IR-6300 instrument (JASCO Corporation, Tokyo, Japan), and the spectra were recorded over the IR spectrum of 400–4,000 cm^{-1}.

2.8.4 NMR analysis

The identity of F1 was confirmed using Nuclear Magnetic Resonance (NMR) spectroscopy (Bruker BioSpin, Rheinstetten, Germany). The ^{1}H NMR spectrometer was operated at 400 MHz, DMSO was used as the solvent of choice, and the spectra were recorded as follows. The ^{13}C NMR spectrometer was operated at 100 MHz, DMSO was used as the solvent of choice, and the spectra were recorded as follows.

2.9 LC-MS/MS analysis

The molecular mass of F1 was determined by LC-MS/MS analysis. 100% methanol and 0.5% (v/v) acetic acid were utilised as solvents (A) (B). The isocratic elution appeared: I used 55% of the solvent A from the start of the run to 10 minutes, 65% from 11 to 20 minutes, and 35% from 21 to 30 minutes. The PDA detector (UPLC LG 500 nm) was seen at 340 nm while the column temperature was maintained at 30 °C. The mass spectrometer (MS) was run in positive ionisation mode with a mass range of 150 m/z to 1000 m/z, a capillary voltage of 3.50 kV, a cone voltage of 30 V, an extractor voltage of 3 V, a gas flow of 30 L/Hr, and a collision gas flow of 0.18 mL/Min.

2.10 Antioxidant activity

Using the DPPH free radical scavenging experiment published by Harsahay Meena et al. [15] and Muddukrishnaiah K et al. [16], the antioxidant activity of the methanolic extract of *Ficu ariculata* fig was investigated. As a reference standard, ascorbic acid was diluted in distilled water to create a stock solution with a concentration of 1 mg/1000 l. Before UV measurements, a fresh solution of DPPH in methanol at a concentration of 60 M was produced. 100 mL of the test solution and different concentrations of this solution (3.9 mL) (200, 400, 600, and 800 g) were combined. The samples were held at room temperature in the dark for 15 minutes while the absorbance was measured. Three duplicates of the experiment were performed. A control sample with the same volume but no extract or reference ascorbic acid was created. As a blank, 95% methanol was employed.

The following formula was used to determine the amount of radical scavenging activity.

% Inhibition = (Absorbance of Control at 0 minute - Absorbance of test) / Absorbance of Control at 15 minutes x 100

Where;
C= absorption of control sample (t= 0 min),
C= absorption of control (t=15 min),
T=absorption of test solution.

3. Results and discussion

3.1 Phytochemical analysis

Qualitative analysis and Quantitative estimation of phytochemicals from *Ficu ariculata*. The present study on *Ficu ariculata* revealed that the presence of active phytochemical constituents was carried out.

Table 1 Qualitative phytochemical analysis of *Ficu ariculata*

S.NO	Extracts	Phytochemicals			
		Flavonoids	Alkaloids	Phenols	Tannins
1	PE	-	-	+	-
2	Cl	+	-	+	+
3	EA	+	-	+	+
4	MeOH	-	-	+	-

(-): Absent, (+): Present

Table 2 Quantitative phytochemical estimation of *Ficu ariculata*

S.NO	Extract	Phenols µg /ml	Alkaloids	flavonoids in µg/ml
1	PE	3.6	18.66	6
2	Cl	24	19.33	134
3	EA	33.8	21.5	144
4	MeOH	7.7	15.16	18

The active phytochemical compounds of Ficus have been analysed qualitatively and quantitatively, and the results are shown in Table 1 and Table 2, respectively.

3.2 Isolation and Identification of bioactive compounds from the methanolic extract

3.2.1 FT-IR spectroscopy

This method is very useful in the process of identification of the functional groups of the phytoconstituents. Purified compound. Purified fraction conducted IR (υmax, cm^{-1}, KBr) spectroscopy analysis, and from the IR data, 3472 (OH, Strong, broad), 2075 (OH, weak, broad), 1641 (C=O Strong) functional groups were identified.

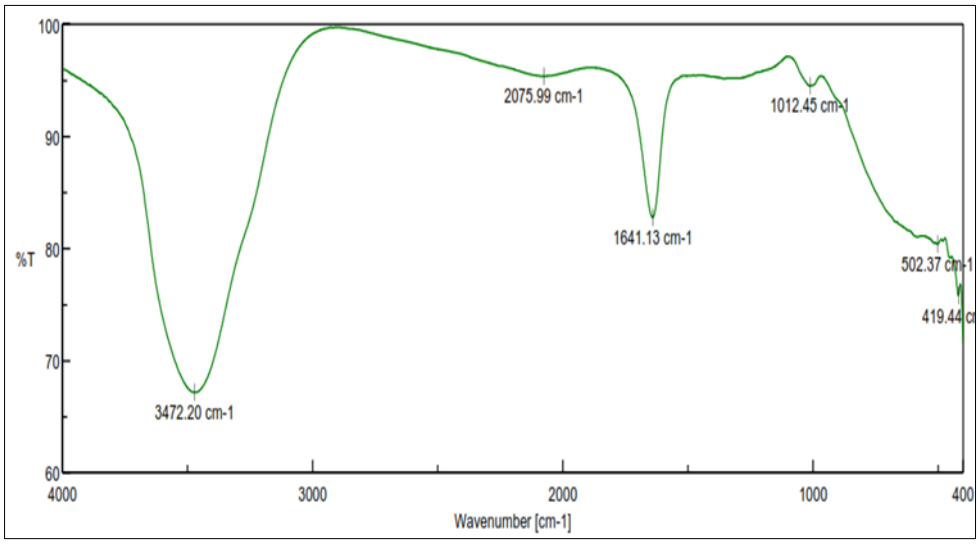

Figure 1 IR range of 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one

3.2.2 LC-MS/MS analysis

LC-MS (ESI) *m/z* (% of relative abundance) calculated for $\mathbf{C_{20}H_{18}O_5}$: 338.35, Found $\mathbf{C_{20}H_{18}O_5^{+1}}$:339.47 [M+1].

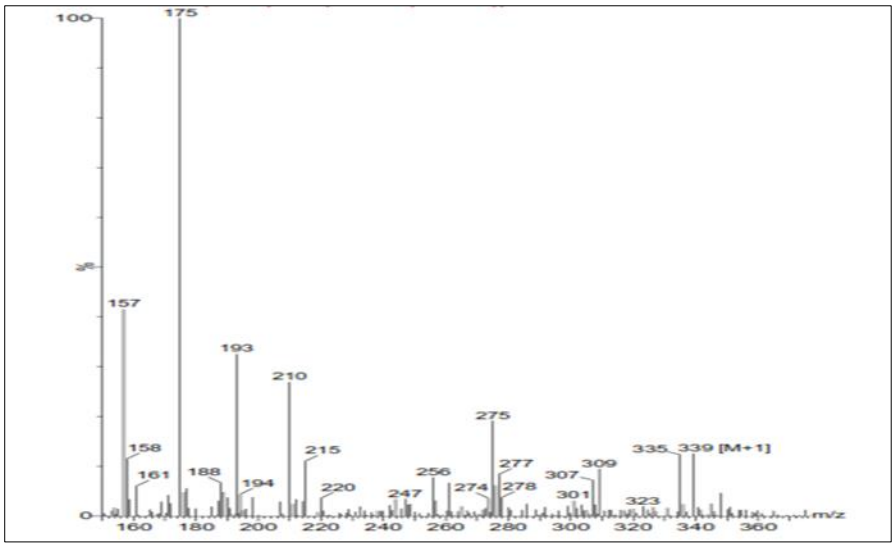

Figure 2 LC/MS data of 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one

3.2.3 HPLC analysis

The purity of the isolated chemical was verified by HPLC using RT 8.47.

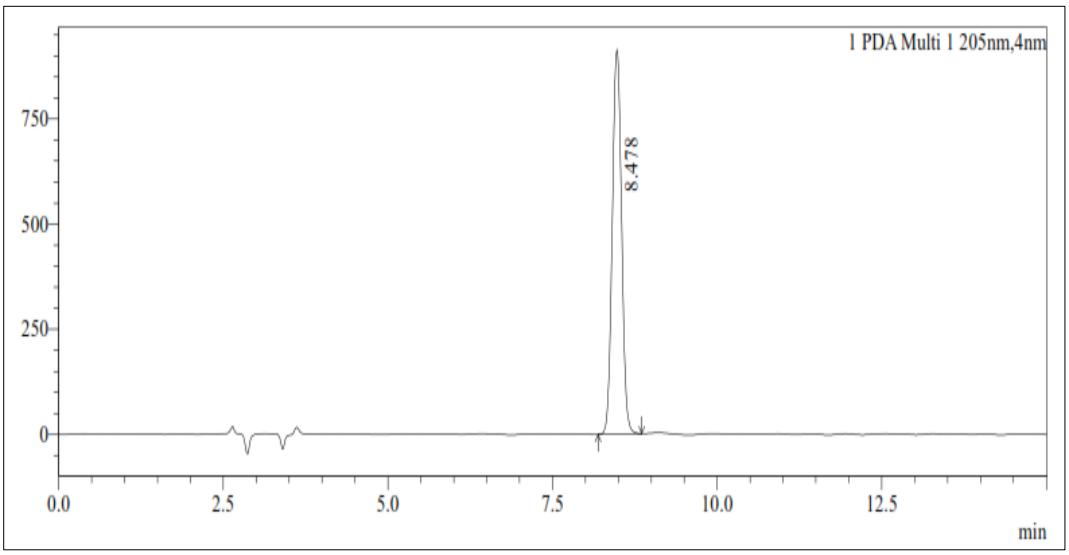

Figure 3 HPLC data of 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one

3.2.4 NMR analysis

- H NMR

^{1}H NMR: δ 1.11 (3H, s, Aliphatic CH_3 substituted in 2H-pyran-4-ol dihydrate (No: 24)), 2.09 (3H, s, Aliphatic CH_3 substituted in aromatic benzyl (No:25)), 2.70-2.97 (2H, dd, *J* = 16.5, CH_2 of Oxan 4-one, No:9), 4.52-5.19 (4H, m, *J* = 2.3 Hz, No: 2,3,6,7 H-atoms), 7.07 (3H, s, No: 16, 20,22 H-Atoms)), 7.33 (1H, s No: 21 H Atom), 10.01 (1H, s, OH, No: 18), 12.97 (1H, s, OH, No: 23).

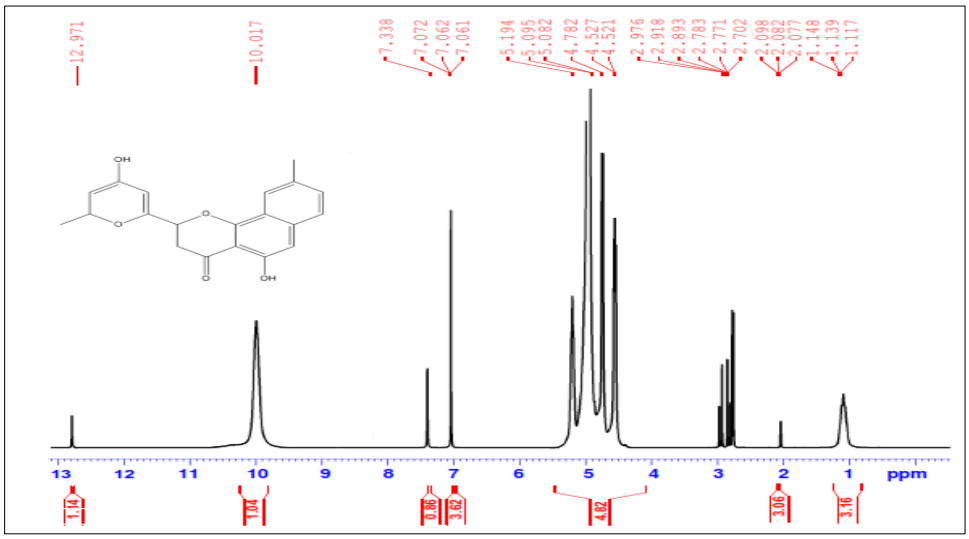

Figure 4 H^{1} NMR of 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one

- 13C NMR

^{13}C NMR (100 MHz DMSO-d6): 192 (1- Carbonyl), 159 (1-Napthalene), 158 ((1-Napthalene)), 153 (1-Napthalene), 152 (1Ethylene), 134 (1-Napthalene), 131 (1-Napthalene), 126 (1-Napthalene), 124, 124 (1-Napthalene), 119 (1-

Napthalene), 105, 105 (1-Napthalene), 103 (1-Napthalene), 89 (1 Ethylene), 80 (Aliphatic C), 70 (Aliphatic C, No:), 35 (Aliphatic C, No:), 20 (Aliphatic C).

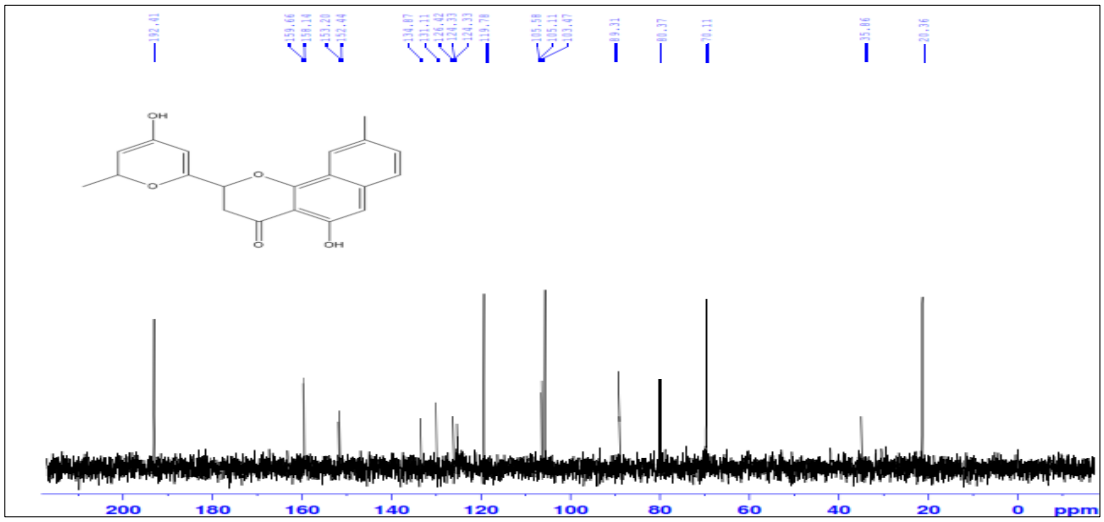

Figure 5 C^{13} NMR of 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one

3.2.5 Spectral Characterization

IR (υmax, cm^{-1}, KBr): 3472 (OH, Strong, broad), 2075 (OH, weak, broad), 1641 (C=O Strong). ^1H NMR: δ 1.11 (3H, s, Aliphatic CH_3 substituted in 2H-pyran-4-ol dihydrate (No: 24)), 2.09 (3H, s, Aliphatic CH_3 substituted in aromatic benzyl (No:25)), 2.70-2.97 (2H, dd, *J* = 16.5, CH_2 of Oxan 4-one, No:9), 4.52-5.19 (4H, m, *J* = 2.3 Hz, No: 2,3,6,7 H-atoms), 7.07 (3H, s, No: 16, 20,22 H-Atoms)), 7.33 (1H, s No: 21 H Atom), 10.01 (1H, s, OH, No: 18), 12.97 (1H, s, OH, No: 23) 13C NMR (100 MHz DMSO-d6) : 192 (1- Carbonyl), 159 (1-Napthalene), 158 ((1-Napthalene)), 153 (1-Napthalene), 152 (1Ethylene), 134 (1-Napthalene), 131 (1-Napthalene), 126 (1-Napthalene), 124, 124 (1-Napthalene), 119 (1-Napthalene), 105, 105 (1-Napthalene), 103 (1-Napthalene), 89 (1 Ethylene), 80 (Aliphatic C), 70 (Aliphatic C, No:), 35 (Aliphatic C, No:), 20 (Aliphatic C). LC-MS (ESI) *m/z* (% of relative abundance) calculated for $\mathbf{C_{20}H_{18}O_5}$: 338.35, Found $\mathbf{C_{20}H_{18}O_5^{+1}}$:339.47 [M+1]. The purity of the isolated compound was confirmed by HPLC with RT 8.47.

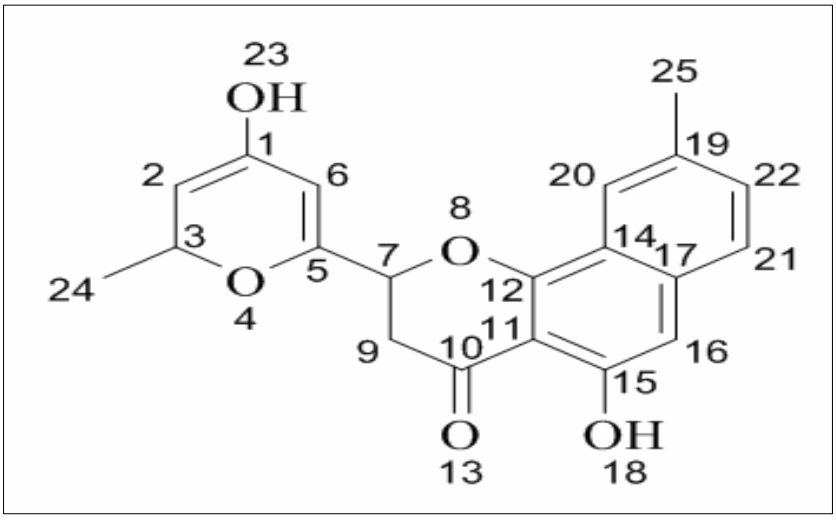

Figure 6 5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one

3.3 Antioxidant activity of Methanolic extract *Ficus ariculata*

Using a significantly modified approach, radical scavenging activity of plant methanolic extract against stable DPPH was measured. An antioxidant reduces DPPH by donating hydrogen. A UV visible light spectrophotometer measured the change in colour at 515 nm. Antioxidant *Ficus ariculata* methanolic extract.

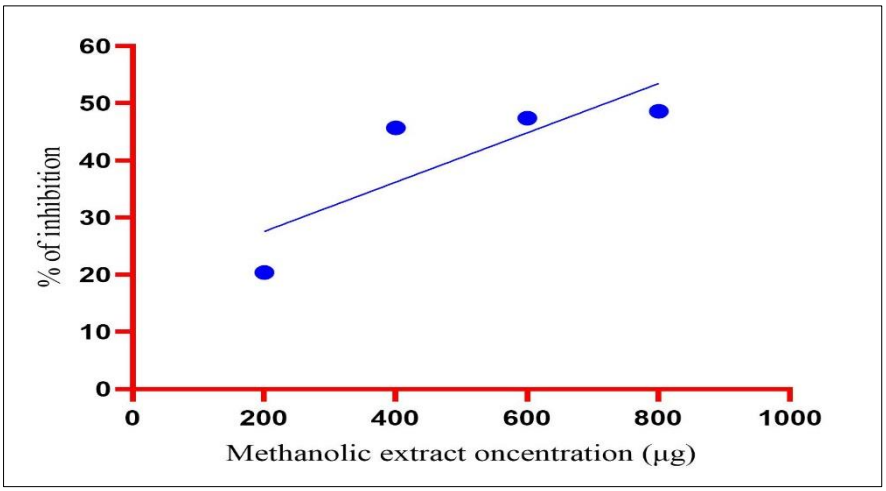

Figure 7 Antioxidant activity of methanolic extract *Ficus ariculata*

4. Conclusion

Plants are major sources of potentially useful structures for developing novel bioactive compounds [17]. *Ficus ariculata* is the famously known fig in the traditional system of medicine with the different pharmaceutical applications. In this work, we have studied qualitative and quantitative analysis of different extracts of *Ficus ariculata* and also isolated and identified novel compound (5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one) form the methanolic extract of *Ficus ariculata* by using chromatographic and spectroscopical analysis. Methanolic extract of *Ficus ariculata* studied antioxidant activity by DPPH method showing good antioxidant activity. From this study finally concluded that *Ficus ariculata* having novel bioactive compound (5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H, 3H, 4H-naphtho[1,2-b] pyran-4-one) and it having good biological activity (Antioxidant activity).

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors declare that no conflict of Interests.

References

[1] Leila Mousavi, Rabeta Mohd Salleh & Vikneswaran Murugaiyah (2018) Phytochemical and bioactive compounds identification of Ocimum tenuiflorum leaves of methanol extract and its fraction with an anti-diabetic potential, International Journal of Food Properties, 21:1, 2390-2399.

[2] Doughari JH, Human IS, Bennade S, Ndakidemi PA. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research. 2009, 3(11), 839–48.

[3] Madziga, H.A.; et al. Phytochemical and Elemental Analysis of Acalypha Wilkesiana Leaf. Journal American Sciences 2010, 6(11), 510–514.

[4] Bhakta P Gaire, Ramakanta Lamichhane, Chitra B Sunar, Amrita Shilpakar, Sabita Neupane and Sushil Panta. Phytochemical Screening and Analysis of Antiacterial and Antioxidant activity of *Ficus auriculata* (Lour.) Stem bark, Pharmacognosy Journal, 2011; 3(21): 49-55.

[5] Yinxian Shi, Huabin Hu, Youkai Xu and Aizhong liu. An ethnobotanical study of the less known wild edible figs (genus Ficus) native to Xishuangbanna, Southwest China, Journal ethnobiology and Ethnomedicine,2014;10(68): 1-10.

[6] Prakash Deep, Amrit Kr. Singh, MD. Tahir Ansari, Prashant Raghav. Pharmacological Potentials of Ficus racemosa-A Review. International Journal of Pharmaceutical Sciences Review and Research, 2013; 22(1): 29-34.

[7] Sharma R.K, Goyal A.K, Yadav S.K, Bhat R. A. Pharmacognostical and Phytochemical Studies of the fruit of Ficus religiosa. International Journal of Drug development and Research, 2013; 5(4): 211-213.

[8] Satish A. Bhalerao and Amit S. Sharma. Ethenomedicinal, Phytochemical profile of Ficus religiosa Roxb. International Journal of Current Microbiology and Applied Sciences, 2014; 3(11): 528-538.

[9] Afroz N, Ahsanul Hoq M, Jahan S, et al. Methanol soluble fraction of fruits of Annona muricata possesses significant antidiarrheal activities. Heliyon. 2019; 6(1): e03112.

[10] Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. The Scientific World Journal. 2017; 2017:1-7.

[11] Boutaoui N, Zaiter L, Benayache F, et al. Qualitative and Quantitative Phytochemical Analysis of Different Extracts from Thymus algeriensis Aerial Parts. Molecules. 2018; 23(2):463.

[12] Siddiqui N, Rauf A, Latif A, Mahmood Z. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). J Taibah Univ Med Sci. 2017; 12(4):360-363.

[13] Singleton V.L., Rossi J.A. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16:144–158.

[14] Ajanal M, Gundkalle MB, Nayak SU. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Anc Sci Life. 2012; 31(4):198-201.

[15] Meena H, Pandey HK, Pandey P, Arya MC, Ahmed Z. Evaluation of antioxidant activity of two important memory enhancing medicinal plants Baccopa monnieri and Centella asiatica. Indian J Pharmacol. 2012;44(1):114-117.

[16] Muddukrishnaiah K, Singh S. Antimicrobial, Synergistic Activity and Antioxidant Studies on Multidrug Resistance Human Pathogen using Crude Extract of *Azadirachta indica* Leaf and *Withania somnifera* Rhizome. J Plant Pathol Microbiol. 2015: S3; 009.

[17] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1-10.