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Study of Phytochemicals, Spectroscopic Analysis and Anti Microbial Activity of High Value Bioactive Compound from Methanolic Extract of Flower of Tilkor (Momoradica Monadelpha)

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ABSTRACT

In this paper an attention has been made on the physicochemical study of methanolic extract of flower of Tilkor (Momoradica monadelpha) made by soxhlet extraction process, phytochemical analysis of the extract, bifurcation, isolation of bioactive components through Thin Layer Chromatography (TLC) as well as column chromatography respectively and the characterisation of isolated compound by using of several spectral analysis such as 1_H NMR, 13_C NMR, IR, U.V. Mass spectroscopy.

In the methanolic extract of flower of the plant (in tropical conditions of Mithilanchal, Bihar, India) the presence of phytochemicals like alkaloids, flavanoids, tannins, saponins, cardiac glycosides, steroids, phenol, protein, lipid, acid carbohydrate etc were reveled. The secondary metaboilities showed Antimicrobial activity. The two compounds isolated from flower were characterised by spectroscopic techniques which revealed the structure of compound A as n – dotriacontan – 15 – one , compound B as n – hexadecanyl oleate.

Keywords: Momoradica monadelpha, Tilkor, Physicochemical analysis, Phytochemical analysis, Isolation, Antimicrobial study, n – dotriacontan – 15 – one and n – hexadecanyl oleate.

INTRODUCTION

Plants are very important for us for our needs such as food, clothes & shelter since from the beginning human race. And thereafter people learnt to use plants to cure and relieve from physical suffering and due to this herbal medicines was considered to be most widely practiced medicine in the word[1]. After that incident some plants were appreciated for the permanent treatment as well as major source of new medicine[2]. These are termed as medicinal plants. Further various investigations and examination have been carried out to identify and characterized the high value bioactive chemical component present in medicinal plants[3-14].

Momoradica Monadelpha (Tilkor) is most popular plant in Mithilanchal, Bihar, India having high Nutritive as well as medicinal value. In Mithila it is employed for preparation of several dishes and medicines. It is grown in India & other oriental countries. All parts of this plant have medicinal value according to herbal practioner or "Baids". Its flower are Antioxidents & Antimicrobials.

The methanolic extract of leaves of Tilkor contain phytochemicals like terpenoid, saponins, tannins, flavonoids, alkaloids and steroids. They also show anti microbial activity and compounds like stigmosterol and tritriacontanet are present in the methanolic extract of leaves of plant.[23]

Mucilage obtained from fruit of the this plant contain phytochemicals like fats, proteins, phytosterols, carbohydrates. The mucilage also shows antimicrobial activity and is very effective against staphylococcus aureus. Compounds like quercetin also found to be present in the mucilage of Tilkor.[25]

So chemical standarisation of the plant seems essential to identify the chemical constituents. The present Investigation was therefore taken up for the physico- chemical study of the methenolic extract of the flower of Tilkor of this region (Mithilanchal, Bihar, India), their phytochemical investigations, antimicrobial examination, isolation of the components present using T.L.C. & column chromatography and their spectral analysis by using various spectroscopic method and also study of its antimicrobial activity.

MATERIALS AND METHODS

1. Chemicals and instruments

All solvents and chemicals are of analytical grade and were used without any purification (Methanol, Silica gel, Calcium sulphate, n- hexane, chloroform, ethyl acetate, petroleum ether, acetone, ethanol, T.M.S., Liquid Na, Chromium (III), acetyl acetone). A soxhelt Extractor is used for the extraction of plant material and separation as well as isolation of components were carried out by thin layer chromatography and column chromatography respectively. IR, UV, Mass, ^{1}H NMR and ^{13}C NMR Spectrometer were used for the spectral characterization of chemical constituents.

Plant material

The flower of plant Momoradica monadelpha [Tilkor] were collected from medicinal plant garden of Shri Himanshu Shekhar Mallik (at Jale) 40 km away from district Darbhanga, Bihar, India.

The part of the plants are authentified by the experts i) Professor Shashi Shekhar Narayan Sinha [International Scientist, Radiation Genetics, Eminent Botanist and Ex. H.O.D. Botany, BRA Bihar University,] ii) Professor (Dr.) Sunil Kumar [Principal, Mahendra. Ayurveda College Tulsipur, Dang, Nepal.]

Preparation of plant extract (Soxhlet extraction)

Fresh flower of Tilkor [Momoradica monadelpha] were washed with distilled water then it shade dried at room temperature. The dried part were then cut and grinded till it get powdered finely.

Now the powdered flowers were then subjected to Soxhlet extractor [914/7] with methanol for continuous hot extraction to get the methanolic extract of flower.

Determination of Physico Chemical Parameter

Physico chemical Parameter such as water and alcohol soluble extractive value, total ash content, moisture content, acid insoluble ash content etc. were determined as per guideline given by WHO.[15]

Phyto Chemical Screening

Preliminary Qualitative and quantative Phyto Chemical Screening for the presence of alkaloid, glycoside, phenol, flavanoid, saponins, tannins, phenol, acid, lipid, protein, carbohydrate, reducing sugar etc. has been carried out by the separated protocol[16-19].

Separation and Isolation

Separation of components obtained from the extract of plant materials were done by "Thin layer Chromatography" (T.L.C.) and isolation of the components was done by column chromotography. T.L.C. was performed on a glass plate of silica gel by the means of standard method[20]

Characterization

The isolated components have been characterised by several spectral characterisation viz – UV, IR, Mass, ^{1}H NMR and ^{13}C NMR spectroscopy.

Antimicrobial Assay

Antimicrobial activity in Methanolic extract of plant sample was determined by agar well diffusion method (NCCL B, 1995). For germinating bacterial strain natural agar was used while potato dextrose agar was used for the growth of fungi. In the process, plant extract disolved in DMSO at concentration of 25, 50, 100, 200 mg/ml.

The reference antibiotic 20 mg/ml concentrated solution of cephaximin were prepared for each bacterial & fungal strain.

EXPERIMENTAL

Physicochemical Parameter

Calibrated digital pH meter was used to determine the pH of 15% and 30% methanolic extract and other physico chemical parameters were determined through standard method[21-24].

Phytochemical screening

Phytochemical screening of the plant sample was done by the means of standard experimental test[19,23,24].

Extraction

The methanolic extract of fine by powdered dried flower of Tilkor was prepared by soxhlet extractor using methanol as solvent (by standard method).

Separation & Isolation: Thin Layer Chromatography (TLC) and Column Chromatography

Methnolic plant extract was taken in a beaker (300 mL) and stirred well for 7 hours. Then the solution was filtered and evaporated using Rotory Evaporator. The residue was dissolved in 20 mL of methanol and the extract(20 μl) was spotted on TLC plate and the colour of spots were recorded. Silica gel – GF 024 391 was used as absorbent. T.L.C. fingerprint profile was developed by using methanol. The column was then eluted successfully with n – hexane and chloroform respectively through column chromatography and hence component were isolated.

CHARACTERISATION

Characterisation of isolated compounds were done by using following spectroscopic techniques U.V. Spectroscopy of sample was done by integrating an optical microscope with U.V. optics, monochromator, white light sources and a sensitive detector.

I R spectrum of sample was recorded by irradicating a beam of infrared light. The amount of light absorbed at each frequency or wave length was measured by the examination of the transmitted light.

The mass fragmentation of the sample was been examined by a mass analyzer and detector. The value of indicator quantity was measured by detector and thus provides the necessary data for the calculation of each quantity present.

^{1}H NMR spectra of sample was recorded in methanol solution and D_2O solvent. TMS was used as reference and chemical shift value for different H – atom was determined.

1 ml plant sample was taken in longer sample tubes (10 nm long in diameter) under high field magnets. Chromium (III) acetyl acetone was taken as relaxation agent and ^{13}C NMR spectrum of sample was recorded.

PHARMACOLOGICAL ACTIVITY

1. Anitmicrobial Assay

The pure culture of pathogenic bacteria & fungi were obtained from department of Microbiology, Darbhanga Medical College & Hospital at Darbhanga, Bihar.
Viz. aggregate bactor actinomycet emcouitians ATCC (12745), Staphylococcus aureas ATCC(10835), Prevotella intermedia [ATCC (225)], Shigella shigella [ATCC (94295)] and Porphyromonas giugiralis ATCC [33658] organism were tested on slant of medium containing 7 mg of nutrient agar / 250 ml. The slant were incubated at temp. 55^0 C for 39 hour and were stored at 10^0 C. The inoculum adjusted at 600 μ m leading to transmission equivalent to 1 x 10 cell /m. The powdered parts of plant were dissolved in DMSO and reference antibiotic cephaximine was prepared. Each plate was inoculated with 20 μ g/ml microbial suspension having concentration of 1 x 10^8 cells. The organisms were tested.

RESULTS AND DISCUSSION

Result

The Physico-chemical analysis's of sample of Tilkor flower is given in Table -1

Table 1. Value of various physico chemical parameters of flower of Tilkor

Physico – Chemical Parameters	Value % (w/w)
Total As	7.2%
Acid insoluble Ash	2.8%
Water Soluble Ash	4.7%
Water Soluble Extracting	9.2%
Alcohal soluble extracting	5.9%

Phytochemical screening: Phytochemical screening of methanolic extract of flower of the plant is given in table no. 2

Table No. – 2 Phytochemical studies of flower of Tilkor

S. No.	Phytochemicals	Test's Name	Result
1	Alkaloid	Wagner's Test	++
2	Saponins	Foam Test	+++
3	Tannin	Lead acetate Test	+++
4	Cardiac glycoside	Legal Test	++
5	Phenol	Standard method	+
6	Protein	Standard method	++
7	Lipid	Standard method	+
8	Acid	Standard method	+
9	Carbohydrates	Standard method	+
10	Flavonoids	Shinoda Test	++

Thin Layer Chromatography (TLC) and Column Chromatography

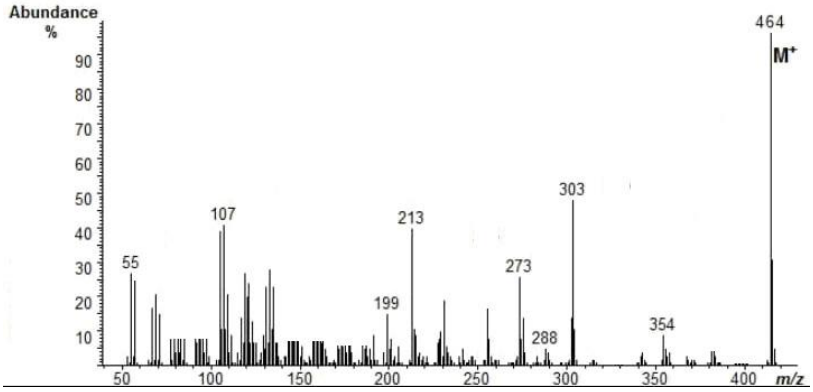
TLC finger print profile was developed by using methanol chloroform & n – hexane solvent in the ratio 2.5:5:7.5 (v/v/v). Five spots in flower extract were observed under UV (of 360nm) light when visualized by using vanillin sulphuric acid Fig.1. Out of which, two compounds were successfully isolated from through the elution with n – hexane and chloroform through column chromatography and were named as compound (A) & compound (B) of flower.

Fig. 1 T.L.C. of flower

Spectroscopic Analysis (characterisation) of compound A of flower extract

Mass Spectroscopy

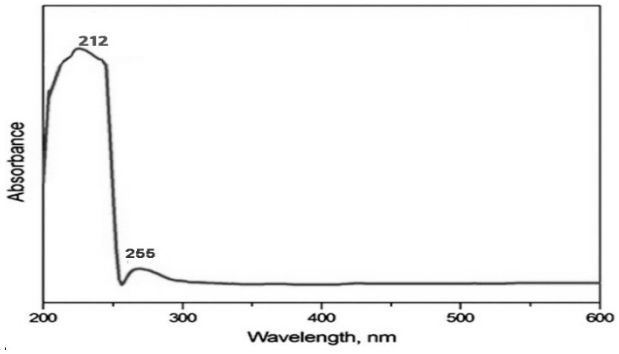
Mass spectrum of compound A shows parent molecular ion [M^+] Peak at m/z 464 which corresponds to molecular formulae $C_{32}H_{64}O$. Other m/z relative intensities are at 55, 107, 199, 213, 273, 288, 303, 354.



Graph No. – 1 Mass Spectrum of compound A

U.V. Spectroscopy

In U.V. spectral analysis λ max (MeOH) was observed to be 212 nm.



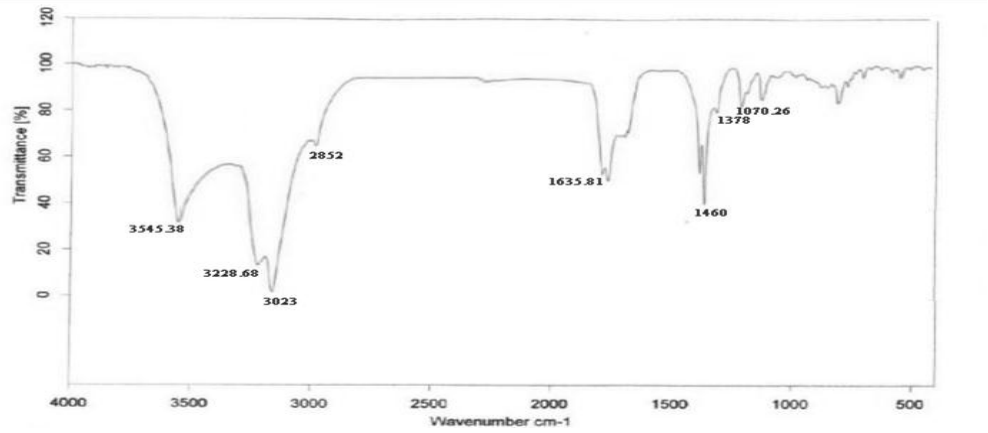
Graph No. – 2 UV spectrum of compound A

I R Spectroscopy

The observed absorption bands of compound A on subjection to IR spectroscopic analysis are given in Table 3.

Table No. 3 The value of IR absorption bands of compound A

s. no.	ν_{max} (KBr) absorption band cum^{-1}
1.	2926 cm^{-1}
2.	2845 cm^{-1}
3.	1705 cm^{-1}
4.	1636 cm^{-1}
5.	1455 cm^{-1}
6.	1370 cm^{-1}
7.	1245 cm^{-1}
8.	1175 cm^{-1}
9.	1059 cm^{-1}
10.	721 cm^{-1}

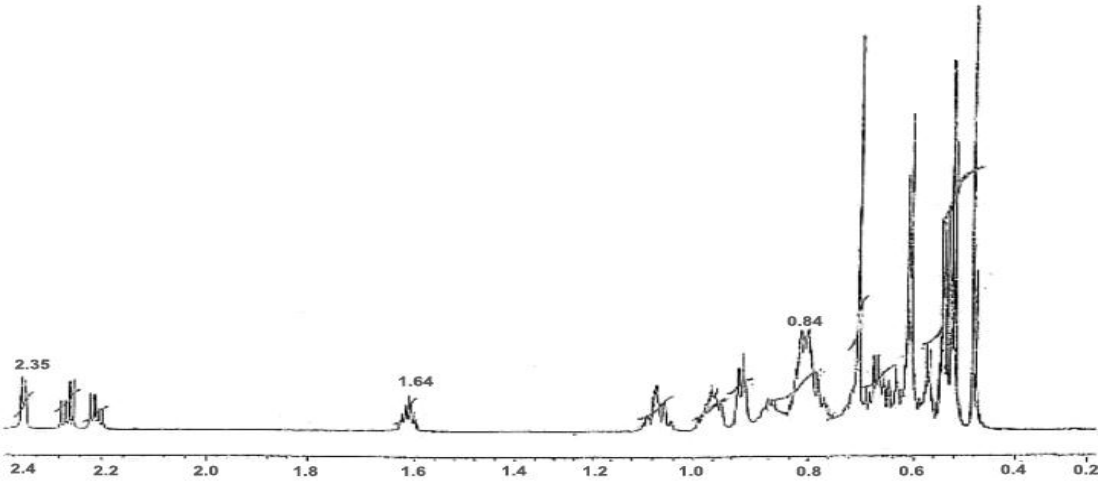


Graph No. – 3 IR Spectrum of compound A

1H NMR Spectroscopy

Table No. – 4 Chemical shift value of ^{1}H NMR of compound A

^{1}H NMR position of H ($CDCl_3$)	Chemical shift value δ
(2H, m, H_2 – 14)	2.35
(2H, m, H_2 – 16)	2.15
(2H, m, CH_2)	1.75
(2H, m, CH_2)	1.64
(2H, m, CH_2)	1.34
(6H, br, s, 3XCH_2)	1.27
(42H, br,s, 21XCH_2)	1.24
(3H, +, J = 65 Hz, Mc - 32)	0.85
(3H, +, J = 65 Hz, Mc - 32)	0.84

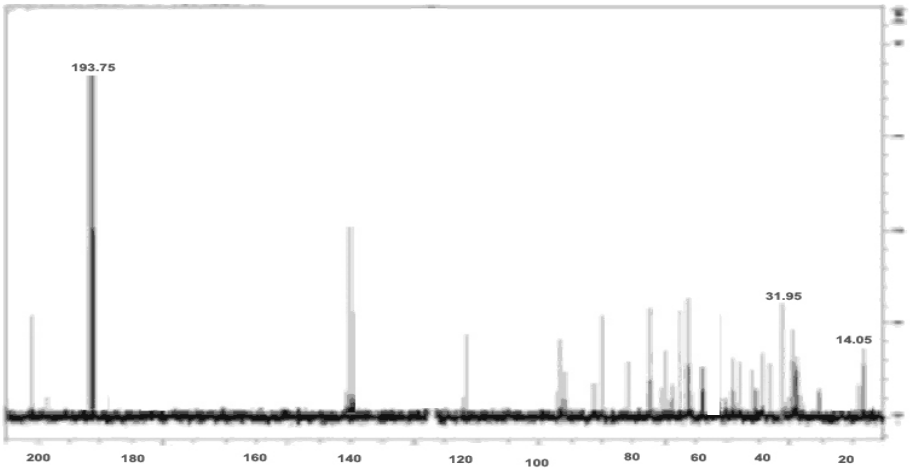


Graph No. – 4 ^{1} H NMR spectrum of compound A

^{13}C ($CDCl_3$)

Table No. – 5 Chemical Shift value of 13C NMR of compound A

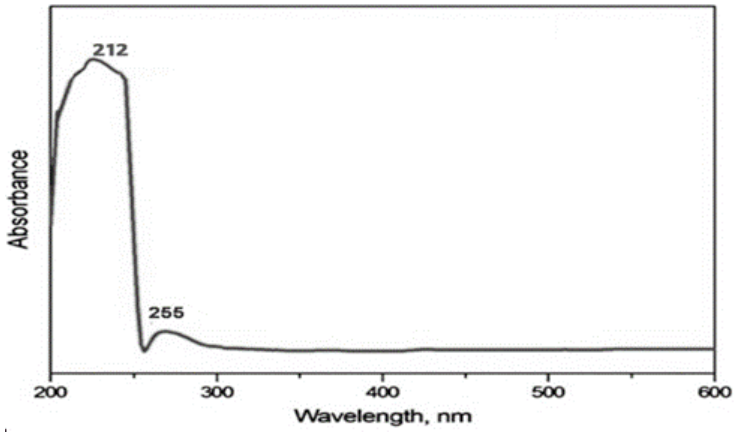
Position & Nature	Value of Chemical shift
C – 15	193.75
CH_2	31.95
26 x CH_2	29.75
CH_2	29.35
CH_2	22.75
Me – 1	14.85
Me – 32	14.05



Graph No. – 5 ^{13}C NMR spectrum of compound A

Spectroscopic Analysis (Characterisation) of compound B of flower extract U.V Spectroscopy

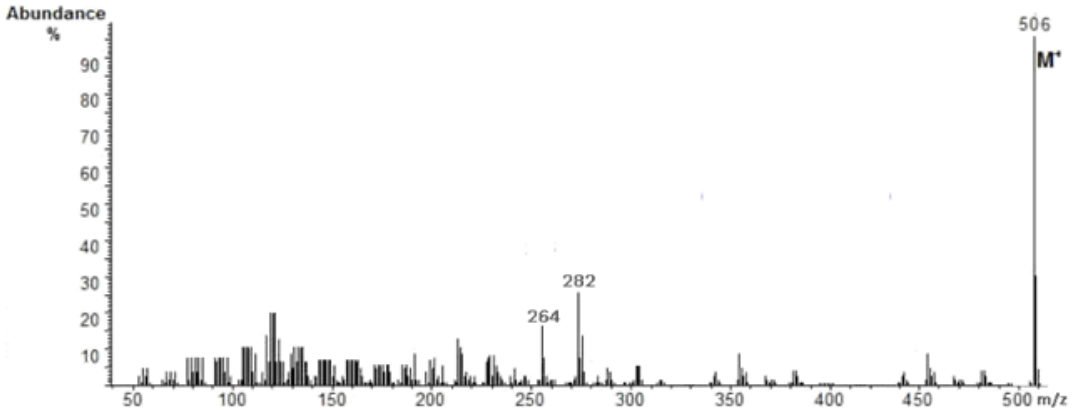
In UV spectroscopy the max value of compound (B) is found to be λ max 212 nm



Graph No. – 6 UV spectrum of compound B

Mass spectroscopy

Mass spectrum of compound B showed parent molecular ion [M^+] peak at m/z 506 which corresponds to the molecular formulae $C_{34}H_{66}O_2$. m/z Rel. Inten – 282 (9.5), 264 (17.1) (Graph No. 7).

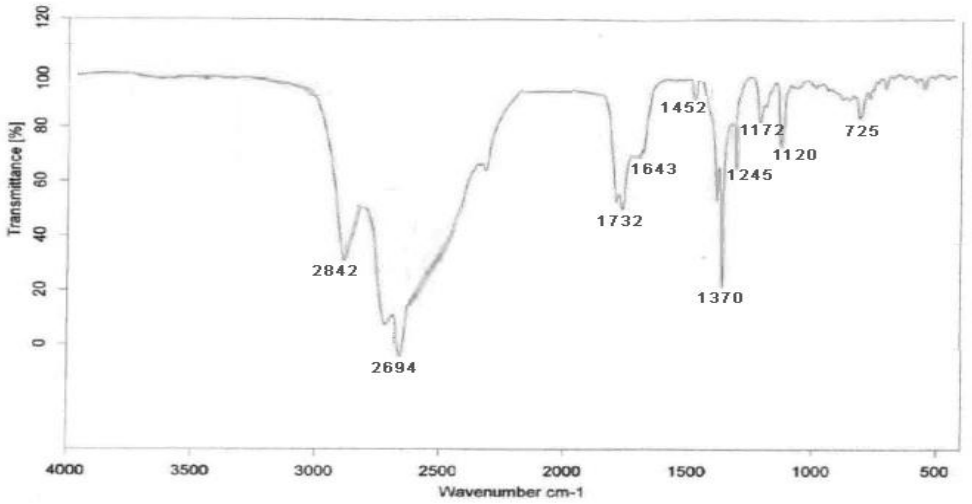


Graph No. – 7 Mass spectrum of compound B

I. R. Spectroscopy

The absorbed absorption band of compound (B) upon subjection to IR spectrometer are given

IR ν_{max} (KBr) 2842, 2694, , 1732, 1643, 1452, 1370, 1245, 1172, 1120, 725 (Graph No. – 8).



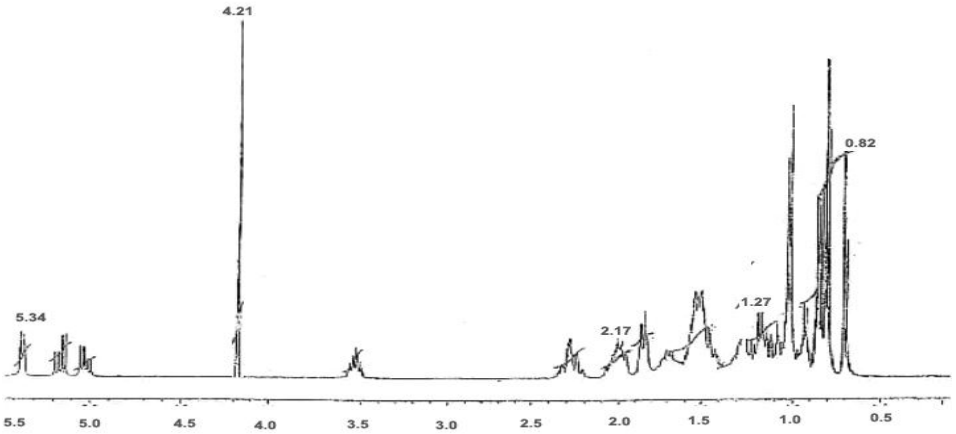
Graph No. 8 IR Spectrum of Compound B

^{1}H NMR Spectroscopy

^{1}H NMR spectroscopic analysis of compound (B) is as

Table No. – 6 Chemical shift value of ^{1}H NMR of compound B

S. No.	Position of H	Value of chemical shift (δ)
1	(2H, m, H – 9 , H – 10)	5.34
2	(2H, t J = 7.3 Hz, H_2 – 1')	4.21
3	(2H, t, J = 7.4 Hz, H_2 – 1')	2.35
4	(2H, m, H_2 – 8)	2.17
5	(2H, m, H_2 – 11)	2.08
6	(4H, m, 2 x CH_2)	1.65
7	(8H, br, s , 4 x CH_2)	1.35
8	(12H, br, s , 6 x CH_2)	1.27
9	(26H, br, s , 13 x CH_2)	1.24
10	(3H, + , J = 6.5 Hz, Me - 18)	0.85
11	(2H, d + , J = 6.3 Hz, Me - 16)	0.82

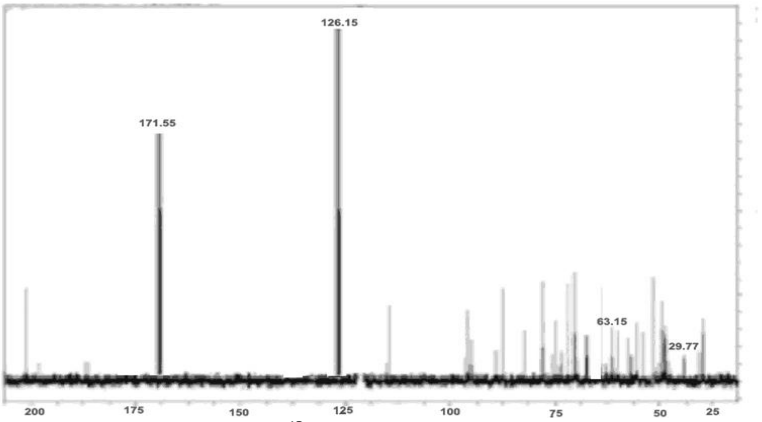


Graph No. – 9 ^{1}H NMR Spectrum of Compound B

^{13}C NMR ($CDCl_2$)

Table – 7 Chemical shift value of ^{13}C NMR of compound B

S. No.	Position/ Natural of carbon	Value of chemical shift
1	C – 1	171.55
2	C – 9	126.15
3	C – 10	123.52
4	C – 1’	63.15
5	CH_2	32.83
6	CH_2	32.05
7	19XCH_2	29.82
8	CH_2	29.77
9	CH_2	29.55
10	CH_2	29.34
11	CH_2	29.17
12	CH_2	27.42
13	CH_2	25.22
14	CH_2	26.67



Graph No. – 10 ^{13}C NMR spectrum of compound B

Pharmacological Activity

The result of antimicrobial activities of flower of Tilkor given in table no. 8 along with pictures of the incubated plates Fig.2.

Table No. – 8 1) Antimicrobial Activity / Assay of flower extract

Sl. No.	Organism	Zone of inhibition (nm) in Conc. mg/ml				Reference antibiotic
		25.0	50.0	100.0	200.0	25 μg /ml
1.	aggregatibacter actinomycetemcomitans	---	----	---	9.6	14.5
2.	Staphylococcus aureus	----	----	8.2	10.5	14.2
3.	Prevotella intermedia	----	----	----	8.7	11.5
4.	Shigella shigella	---	----	----	8.5	12.1
5.	Porphyromonas gingivalis	----	----	13.2	15.2	12.8

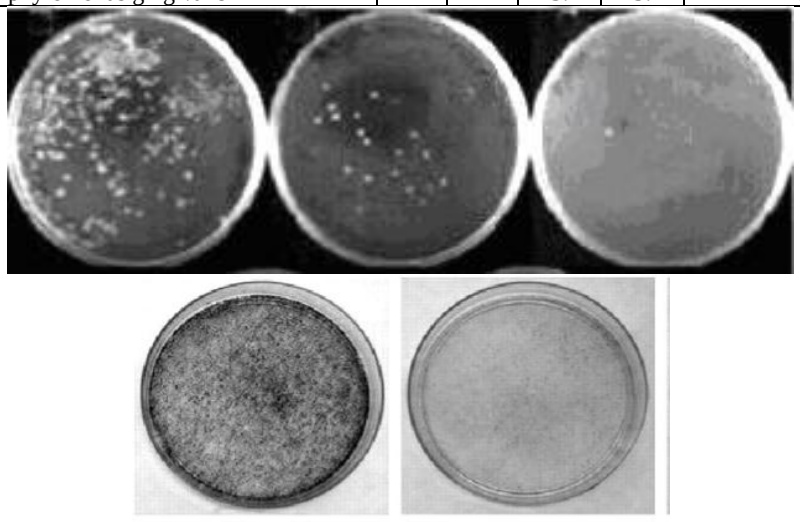


Fig.2 Incubated plates of culture of flower of Tilkor extract.

DISCUSSION

The results of physiochemical studies of flower sample of the plant to determine the total ash, acid insoluble ash, water soluble ash, water soluble extractive, alcohol soluble extractive were given in Table [1].

These results reveled that in the flower sample the % of water soluble ash (47) is greater in comparison to Acid insoluble ash (2.8). Among water and alcohol soluble extracives the % of water soluble extractive (9.2%) is greater than the % of Alcohol soluble extractive (5.9%). Phytochemical screening of mathanolic extract of flower of the plants reveled the presence of alkaloids, saponins, tannins, cardiac glycoside, phenol, protein, lipid, Acid, carbohydrates, and Flaranoid in the extract.

In methanolic extract of flower, saponin and Tannin showed height degree of precipitation (+++). Alkaloids, glycosides, protient and flavanoid showed moderate degree of precipitation (++) and phenol, lipid, acid and carbohydrate showed lower degree of precipitation (+).

Separation and isolation of plant using T.L.C. and column chromatography by the described method visualized under U.V. showed 5 spots in flower extract sample out of which two component were successfully eluted by n – hexane and chloroform through column chromatography. They are named as compound A and B of flower.

Compound A isolated from flower extract of the plant was isolated as colourless powder which showed its IR absorption bands for carbonyl group at 1705 nm^{-1} and long aliphatic chain at 721 cm^{-1}. In UV spectral analysis its value was observed to be λ max 212 nm. Mass spectrum give molecular ion peak at m/z 464 which corresponds to the molecular formulae of aliphatic ketone $C_{32}H_{64}O$. m/z relative intensity at 224 [C_{15} – C_{16} Fission, $CH_3(CH_2)_{12}$] + and 178 [C_{14} – C_{15} fission, CH_3 $(CH_2)_{12}$] + indicates the presence of carboxyl at C_{15} carbon.

The ^{1}H NMR spectrum showed five two proton multiplate from δ 2.35 to δ 1.34 and two broad singlet at δ 1.27 (6H) and 1.24 [42H] assign to methylene protons. Two three proton triplets at δ 0.85 (J=6.5Hz), 0.84 (J=6.2 Hz) were accounted to terminal C – 32 and C – 1 primary methyl protons respectively. ^{13}C NMR spectrum of the sample showed the signal for carbonyl carbon at δ 193.75 (C – 15), methylene carbon between δ 31.95 to δ 22.75 and methyl carbons at δ 14.85 (Me – 1) and 14.05 (Me – 32). The absence of any type of signal in ^{13}C NMR spectrum between δ 193.75 - δ 31.95 and in the ^{1}H NMR spectrum beyond δ 2.35 ruled out the unsaturated nature of the molecular. On the basis of above spectral data analysis, the structure of compound (A) has been concluded as

***n* – dotriacontan – 15 – one.**

Compound B isolated from flower extract of the plant was semisolid. Which showed its IR spectrum based for carbonyl at 1732 cm^{-1} as well as unsaturation at 1643 cm^{-1} and long

aliphattic chain at 725 cm^{-1}. In U.V. spectral analysis its value was observed to be λ max 212 nm. The mass spectrum show parent m/z molecular ion peak at 506 which corresponds to the molecular formulae $C_{34}H_{66}O_2$, m/z relative intensities at 282 and 264 indicates the presence of carbonyl C_1 carbon and unsaturation at 9 carbon (C_9). ^1H NMR spectrum showed 5 two proton multiplate from δ 2.08 to δ 5.34 and four broad singlet at δ 1.65 (4H), δ 1.35 (8H), δ 1.27 (12H) δ 1.24(26H) assign to methylene protons. Two three proton triplet at δ 0.85 (J = 6.5 Hz) and δ 0.82 (J=6.3) which accounted to terminal C – 18 and C – 16' primary methyl protons.

^{13}C NMR spectrum of the sample showed signal for carbonyl carbon at δ 171.55 (C = O), methylene carbons between δ 6.67 to δ 32.83 and methyl carbon at C_{18} and C_{16}'.

From the analysis of above spectral data compound B is charactersised as

***n* – Hexadecanyl oleate**

18 10 9 1 1' 16'

$CH_3(CH_2)_7\ CH = CH\ (CH_3)_7CO – O – CH_2\ (CH_2)_{14}\ CH_3$

Antimicrobial assay of methanolic extract of flower of the plant exhibit higher antimicrobial activities at 200 mg/ml conc. extract against P. gingivalis 15.2 nm in flower extract as compared to reference antibiotic. Antimicrobial activities against other test organism is very less in comparision to reference antibiotic.

CONCLUSION

The methanolic extract of flower of the plant (in tropical conditions of Mithilanchal, Bihar, India) reveal the presence of phytochemicals like alkaloids, flavonoids, tannins, saponins, cardiac glycosides, steroids, phenol, protein, lipid, acid, carbohydrate etc.
The secondary metabolites shows pharmacological activity such as Antimicrobial.

The two isolated compounds from the extract were characterised by spectroscopic techniques which revealed the structure of compound A from flower as n – dotriacontan – 15 – one , compound B from flower as n – hexadecanyl oleate.

ACKNOWLEDGMENT

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REFERENCES

1. Fransworth NR; Akerele O.; Bingel AS; Soejarto DD; Guo Z., Medicinal plants in therapy. Bulletin of the World Health Organization; 63 (6): 965-981 (1985).

2. WHO, general guidelines for methodology on research and evaluation of traditional medicines. HO/EDM/TRM/2000. I Geneva. pp. 74 (2000).
3. Agrawal SS ; Paridhani M. Herbal Drug Technology Univ. Press Pvt. Ltd., Hyderabad, pp. 83, 625 (2007)
4. Kokate C.K.; Purohit A P; Gokhle SB, Pharmacognosy, Nirali Prakashan Delhi, pp. 1 – 2 , 90, 99, 106, 597 (2004).
5. Chaudhary N; Sekhon BS. An overview of advance in standardization of herbal drugs. *Journal of Pharmaceutical Education and Research*, 2(2) 55 – 70 (2011).
6. Al-Snafi AE. Chemical constituents and pharmacological effects of Citrullus colocynthis - A review. *IOSR Journal of Pharmacy*; 6(3): 57-67 (2016).
7. Al-Snafi AE. Medical importance of Cichorium intybus – A review *IOSR Journal of Pharmacy*; 6(3): 41-56 (2016).
8. Al-Snafi AE. Pharmacological importance of Clitoria ternatea – A review *IOSR Journal of Pharmacy*; 6(3): 68-83 (2016).
9. Al-Snafi AE. The medical Importance of Cicer arietinum - A review *IOSR Journal of Pharmacy*; 6(3): 29-40 (2016).
10. Al-Snafi AE. The pharmacological activities of Cuminum cyminum - A review. *IOSR Journal of Pharmacy*; 6(6): 46-65 (2016).
11. Al-Snafi AE. The pharmacological importance of Aloe vera- A review. *International Journal of Phytopharmacy Research*; 6(1) : 28-33 (2015).
12. Shinde VM, Dhalwal K, Potdar M &Mahadik KR. Application of quality control principles to herbal drugs. *International Journal of Phytomedicine*. 1, 4–8 (2009).
13. Mukherjee P K, Quality control of herbal Drugs, 1st Edn Bussiness Horizons Pharmaceuticals Publishers. New Delhi, pp. 131 – 219 (2002).
14. Chandrasekaran M. and Venkatesalu V. Antibacterial and antifungal activity of Syzygium jambolanum seeds. *J Ethnopharmacol*, 91, 105-108 (2004).
15. World Health Organisation, General guidelines for methodology medicines. World Health Organisation, Geneva (2011).
16. Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, 29, 152-178 (1999).
17. Horborne, JB, Phytochemical Method. A guide to modern techniques of plant analysis. 3rd edition, Springer (India) Pvt Ltd., New Delhi, pp. 5-12 (1998).
18. Khandelwal KR, Practical Pharmacognosy techniques and experiments. Nirali Prakashan, Delhi, 19th Edition, Appendix 1, pp. 182 (2008).
19. S Sashidharan, Y Chein, D. Saravanan, K. M. Sundaram, L Yoga Latha, Extraction Isolation and characterisation of bioactive compounds from plant extract. *African Journal of Complement Altern. Med*., 8 (1), pp. – 1 to 10 (2011).
20. Justus G Kichan. Thin Layer Chromatography, 2nd Edition Willey (1978).
21. Johansen DA, Plant – Modern Techniques. 1st Edn. (MC Graw – Hill Book company New York & London), 182 – 203 (1940).

22. Harbone JB, Methods of Extraction & isolation, In phytochemical Methods. Chapman & Hall, London, pp. 60-66 (1998).
23. Pratik Panshu; Mishra P.M., Study of isolation, characterization and antimicrobial activity of high value bioactive compounds from methanolic extract of leaves of Tilkor (Momoradica Monadelpha). *Oriental Journal of Chemistry*, Vol. 34(5) pp. 3145-3152 (2018).
24. Pratik Panshu; Mishra P.M., Superficial fluid extraction, identification and characterization of high value bioactive compounds from medicinal and aeromatic herbs alongwith study of their antimicrobial activity. *Journal of Scientific and Technical Research*, Vol. 8(2), pp. 21-24 (2018).
25. Pratik, Panshu; Shadique, Md. Athar, Antimicrobial examination of Mucilage obtained from fruit of Tilkor (Momoradica Monadelpha): A potential medicinal plant. *J. Biol. Chem. Chron.* Vol. 6(1), 05-09 (2020).