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Original Article

Studies on Antibacterial Compounds from Methanolic Extract of Bark of *Phoenix Dactylifera* and Its Applications

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ABSTRACT

The study evaluates the antimicrobial potential of methanol extract of Date palm bark and to identify potential natural sources for the synthesis of new drugs to address the alarming proposition of growing antimicrobial resistance over the course of last three decades. The crude methanol extract of Bark of Date palm, Phoenix dactylifera was extracted and antibacterial activity was evaluated by paper disc method showed enterprising potential zone of inhibition of 17 mm against S.typhi and K.pneumoniae leading to the further investigation using agar cup method on various standard strains demonstrated 22mm zone of inhibition by Shigella. MIC of E.coli found in the range of 3.12 mg/ml and 0.78mg/ml for S.aureus. The **MBC** for E.coli of Bark crude methanol extract showed 1.25mg/ml and for S.aureus is 1.5mg/ml. Bark Methanol fraction exhibited excellent activity against AST of Activity guided fraction with a zone of inhibition of 20 mm against E.coli and 22mm against S.aureus. Phytochemical analysis reveals the presence of glycoside, saponins, alkaloids, flavonoids and tannins. Characterization of bioactive compounds from the potent bark methanol fraction analysed by HPTLC, followed by bioautography, CHNS, FTIR, LCMS and GCMS analysis profiling the presence of **Lup-20(29)-en-3-ol-acetate,(3α)** may the bioactive compound reporting antibacterial, antiviral and antioxidant activity. The present study also reveals the potential antioxidant activity (12.08mg/ml), MIC of antiviral activity is 780µg/ml and antiviral activity is 99.29 % against tested coli phage. The phage inactivation kinetics indicates 70% inactivation at 20min of exposure. The methanol bark fraction showed a strong ability to inhibit the infectivity of coli phage and completely prevented bacterial lysis, which it is hoped will promote research into its potential as a novel antiviral agent against pathogenic human viruses. Bark methanol fraction showed an excellent antibacterial activity ananist MDR pathogens in the range 12-14 mm of zone of inhibition revealing its potential as defence tool against array of microbes.

Keywords: P.dactylifera, Antibacterial Activity, Phytochemical, HPTLC, GCMS, LCMS, FTIR and CHNSO, Antioxidant Activity, Antiviral Activity

1. INTRODUCTION

Antimicrobial resistance is a major menace encountered by the present day scientific community and the world at large, as the number of multi-resistant pathogenic bacterial strains has grown exponentially and assumed alarming proposition over the course of

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last three decades. **"Green medicine"** is increasingly gaining importance and garnering world- wide attention as the global focus has shifted to traditional medicines. The fruit of the date palm (Phoenix dactylifera) is an important commercial crop in Middle East countries. The date palm is a monocotyledonous woody perennial fruit species belonging to the Arecaceae family (Mc Clintock, 2007) called 'Nakhla' and the 'Tree of life' by the Arabs and is considered as one of the oldest cultivated fruit trees. Many Middle Easterners believe that consumption of date fruits, particularly in the morning on an empty stomach, can reverse the actions of any toxic material that the subject may have been exposed to. Different parts of this plant are traditionally claimed to be used for the treatment of a broad spectrum of ailments including memory disturbances, fever, loss of consciousness and nervous disorders. Dates are good source of energy, vitamins and a group of elements like phosphorous, iron, potassium and a significant amount of calcium (Anwar-Shinwari, 1987: Gamil-Abdel-Hafez, Fouaud-Shalaby, & Akhal, 1980). Besides nutritional values, date fruits are rich in phenolic compounds possessing antioxidant activity.[1-3]

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. (Srivastava et al .1996). A wide range of medicinal plant parts is used as extract for raw drugs and they possess varied medicinal properties. WHO estimated that 80% of the people world-wide rely on plant based medicines for their primary health care (Alagesaboopathi, 2011). As the global interest towards traditional medicines over the conventional treatment is increasing due to their safer action (in terms of tolerance and side-effects) for chronic illnesses, this study is undertaken to evaluate antibacterial, antiviral, antioxidant properties of some cultivars of date palm of Middle East countries, which may be developed into new, safer and more efficacious agents to combat serious microbial infections.

2. EXPERIMENTAL

Extraction of Date Palm Bark by Alade and Irobi's cold extraction Method: 20gm powder of bark was taken separately in 200 ml methanol and kept for 72 hours at room temperature and stirred with a glass rod after every 24 hours .After 3 days, the mixture was filtered using the Whatman's filter paper no:1. The filtrate was air dried to remove solvent and concentrated in Rotary Vacuum Evaporator. The concentrated extract was stored in the refrigerator (4^0 C) for further use. (Perveen Kahkashan, 2012) [4-6]

Microorganisms:

Reference bacteria strains were obtained from Microbiology department, Bhavan's college. The test culture includes - Normal flora 1. Escherichia coli NCIM 2641 Human pathogens- 1. Staphylococcus aureus MTCC 1144 2. Klebsiella pneumonia MTCC 4032 3. Salmonella typhimurium NCIM 2501 4. Shigella flexneri MTCC 1457 5. Vibrio cholerae MTCC 3906 6. Proteus vulgaris NCIM 2813 7. Salmonella paratyphi A MTCC 3220, 8.Salmonella paratyphi B, 9. Streptococcus pyogenes, Pseudomonas aeruginosa, and Proteus vulgaris. The strains were maintained on agar slant at 4^0c and activated at 37^0c for 24 hours on nutrient agar media. [7-10]

I. Antibacterial Assay :- (Al-daihan Sooda2012) Antimicrobial susceptibility test (AST) was used to determine the efficacy of potential antimicrobials from biological extracts against human pathogens. The crude methanol extracts of the **Date Palm Bark** were subjected to antimicrobial assay using:-

a) Paper disc diffusion method
b) Agar cup method/Agar well diffusion method

II. Minimum inhibitory concentration: (Mathur Rashmi, 2013)

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The turbidometric method or tube dilution method was used for determination of minimum inhibitory concentration, minimum bactericidal concentration, and minimum fungicidal concentration. [11- 13]

a) Determination of Minimum Inhibitory Concentration (MIC)
b) Determination of minimum bactericidal Concentration (MBC)

III. Activity guided fractionation:

For successful isolation of the bioactive compounds from the **Date Palm Bark**, the crude methanol extracts were sequentially fractionated with various organic solvents differing in their polarity, from highly polar to non-polar, and each obtained fraction subjected to bio assay. (Mathew et al, 2014) [14, 15]

IV. Phytochemical analysis:

Phytochemical analysis was performed by various qualitative tests to find the phytoconstituents present in them. (Egon, Wagner)

a) Chemical tests by tube method were carried out by using all the fractions of extracted by the process of activity guided fractionation and to identify the constituents present in them.
b) Phytochemical analysis by HPTLC method for detection of class of compound.

V. Characterization of Potent Date Palm Bark Methanol Fractions: -

a) Detection of Bioactive compounds by Bioautography (Choma 2015), Bioautography is a means of target- directed isolation of active molecules on chromatogram. Organic solvents employed in chromatographic separation process can be completely removed before biological detection because these solvents can cause inactivation pf enzymes and/ or death of living organisms
b) Isolation and identification, of antimicrobial compounds includes HPTLC (Mahesh Attimarad 2011), CHNS, FTIR, LC-MS, and GC-MS analysis.

VI. Determination of Antioxidant activity of Date Palm Bark Methanol fractions by Phosphomolybdenum method (Ibrahim, 2012) Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Total antioxidant capacity can be calculated by the method described by Prieto et al. (1999). [16, 17]

VII. Determination of Antiviral activity of Date Palm Bark Methanol fractions

a) **Determination of minimum inhibitory concentration value:**

The MIC of the **Bark Methanol fractions** was determined for test coli phage of E. coli as follows: The Microdilution method using 96 well microliter plates described by the National Committee for Clinical Laboratory Standards (NCCLS) was used. (Jassim Sabah, 2010) [18]

b) **Phage inactivation Assays:** (Adams1959)
c) **Determination of Phage Inhibition Kinetics:** (Jassim sabah,2010)

VIII. Evaluation of antimicrobial activity of most potent fraction against some MDR pathogens: Agar cup diffusion method performed using sterile Mueller Hinton Agar, MDR strains and using standard antibiotics as control. (Sharifi Javad 2013) [19-24]

3. RESULT AND DISCUSSION

Paper disc method, a routine preliminary sensitivity testing used to determine the anti-bacterial activity of the methanol date palm bark crude extract which demonstrated 17mm of zone of inhibition by both K.pneumoniae and S.typhi showed the promising

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activity other than Gram negative bacteria. (Sabah et al., 2007; Ammar et al., 2009)

Fig1: Date Bark

Fig2: Cold extraction method

Fig 3: Antibacterial activity by Paper disc method

Fig 4: Antibacterial activity by Agar cup method

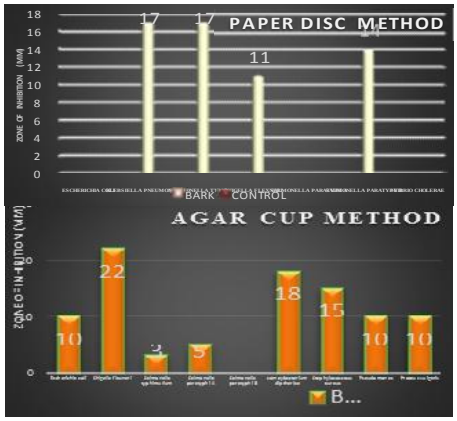


Figure: 5 & 6 Graphical representation of Antibacterial activity of crude extract of Phoenix dactylifera Date palm Bark by Paper Disc Method & Agar cup Method

Antibacterial property of the crude bark methanol extracts was evaluated against 12 pathogenic organisms using agar cup method which showed zone of inhibition in the range 22mm by Shigella. (Sooad Al-daihan and Ramesa Shafi Bhat, 2012).

The MIC for E.coli was found as 3.12mg/ml and for S.aureus in the crude methanol bark exhibited 0.78mg/ml. The MBC for E.coli of Bark crude methanol extract showed 1.25mg/ml and for S.aureus is 1.5mg/ml (Kahkashan Perveen, Najat A. Bokhari, 2012).

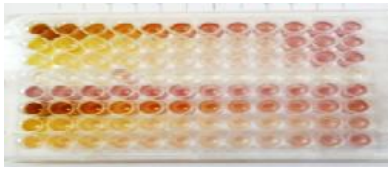


Fig:7 Antibacterial activity of crude methanol bark extract by MIC

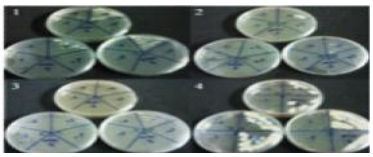


Fig:8 Antibacterial activity of crude methanol bark extract MBC

The crude extract was subjected to **activity guided fractionation** and the fractions underwent bioassay and the bark methanol fraction showed maximum zone of inhibition of 20 mm against E.coli and 22mm against S.aureus (Cragg et al, 1996). It was observed that Staphylococcus aureus was not much sensitive in crude extract but were very sensitive in methanolic fraction. This indicates that there might be some phytocompound soluble in this fraction that may be potent against the organism. Escherichia coli also showed its sensitivity in methanolic fraction as compared to acetone and ethyl acetate. The zone of inhibition shows the distribution of antimicrobial activity is less in nonpolar fractions indicating that the active compounds are more polar in nature.

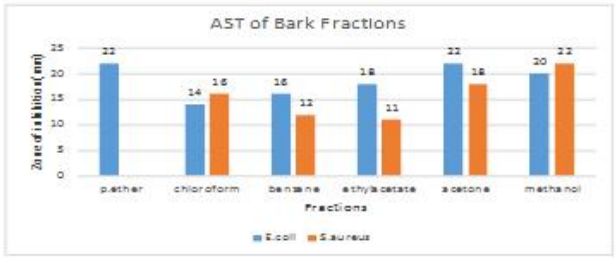


Fig: 9 Graphical representation of AST of Date Palm Bark Fractions

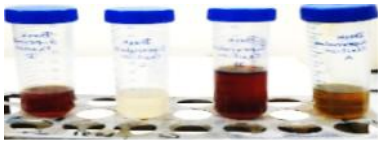
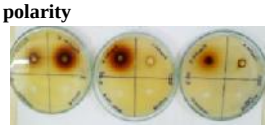


Fig 10: Fractionation of bark with different solvents varying in polarity



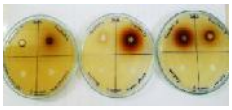


Fig: 11, 12: AST of each fractions of bark – E.coli & S. aureus with control

Phytochemical analysis of bark methanol fractions (Chemical Tests by Tube Method**)** revealed the

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presence of phytoconstituents like glycoside, saponins, phytosterol, alkaloids, anthraquinone, flavonoids and trepenoids except tannins and coumarins. (Sooad Al-daihan and Ramesa Shafi Bhat, 2012). Phytochemical analysis for detection of class of compounds by **HPTLC profiled** the levels of glycosides, alkaloids, flavonoids, tannins, and saponins.

Then the potent bark methanol fractions was taken for further characterization of compounds. Based on the results of HPTLC for detection of class of phytochemical compound, the solvent system which yielded better seperation as follows: Toluene: Chloroform: Ethanol in the ratio 8:8:2.(20,24)

The **HPTLC** spectra for **bark** methanol fraction showed 8 peaks that is the presence of 8 different class of phytochemical compounds at a wavelength of 254nm with Rf value ranging from Rf 0.09 to Rf 0.80. (Tambe Rashmi, Maushumi Kulkarni, 2013) The peak 7 that is with an Rf value of 0.65 showed sharp peak with an area of 10686.8 and an area% 29.87 at 254nm. There are 6 autogenerated peaks showed at 366nm. Peak 5 with Rf value of 0.64 is a well defined sharp peak showing the highest area of 29332.3 with an area % of 81.24 at 366nm. More peaks are present at 540nm , peak 10 showing highest peak area 12165.9 with an area % 21.85.

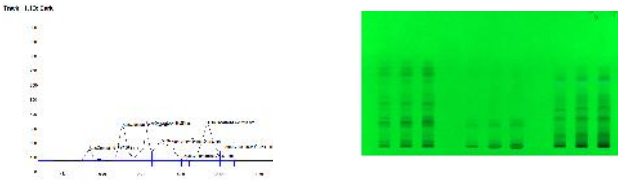

Fig13:Graphical representation of HPTLC result of Bark Methanolic fraction spectra and image of derivatized TLC plate at 254nm

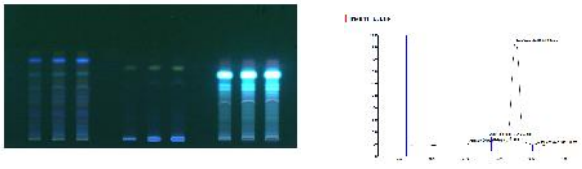

Fig14:Graphical representation of HPTLC result of Bark Methanolic fraction spectra and image of derivatized TLC plate at 366nm

Table 1: Result of HPTLC of Bark Methanolic Fraction at 254 nm ,366nm and 540nm

Peak @ 254nm	Rf @254 nm	Area	Area % 254nm	Peaks @ 366nm	Rf@366 nm	Area	Area % 366nm	Peak @ 540	Rf @ 540	Area	Area % 540nm	Bioautography result of coinciding Rf values
1	0.09	1715.1	4.79	1	0.40	473.8	1.31	1	0.06	1183.7	2.13	
								2	0.13	271.8	0.49	
2	0.25	6749.2	18.87	2	0.45	1211.7	3.36	3	0.18	312.1	0.56	
3	0.36	5543.8	15.50	3	0.52	1347.7	3.73	4	0.26	2581.7	4.64	0.25
4	0.46	5500.4	15.38					5	0.37	3981.3	7.15	
5	0.54	2722.4	7.61	4	0.56	3353.7	9.29	6	0.42	11006.9	19.76	
6	0.61	257.1	0.72	5	0.64	29332.3	81.24	7	0.51	7318.5	13.14	
7	0.65	10686.8	29.87					8	0.55	10935.5	19.64	0.57
8	0.80	2599.5	7.27	6	0.81	386.2	1.07	9	0.64	5932.9	10.65	0.61
								10	0.74	12165.9	21.85	0.74

Fig15:Graphical representation of HPTLC result of Bark Methanolic fraction spectra and image of derivatized TLC plate at 540nm

Out of 10 peaks from bark methanol fraction at 540nm, four peaks are coinciding against **bioautography** values as follows: peak 4 with Rf- 0.26 coincides with Rf- 0.25, peak 8 with Rf- 0.55 coincides with Rf- 0.57, peak 9 showing Rf- 0.64 corresponds to Rf 0.61 and Rf- 0.74 coincides with the corresponding Rf-0.74. (7,20)

Fig 16: Bioautography result of bark methanolic fraction

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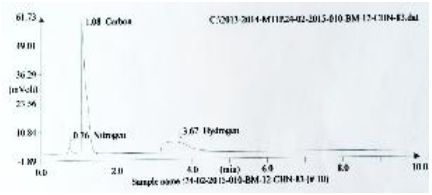

Fig 17: CHNS spectra of Bark methanolic fraction

Table 2: Result of CHNS Analysis of Bark methanolic fraction

Peak No.	Retention Time (min)	Area (0.1*uV*sec)	Element %	Component
1	0.758	194915	3.263	Nitrogen
2	1.083	4787943	38.581	Carbon
3	3.667	2618950	7.391	Hydrogen

FTIR analysis of Bark methanol Fraction is carried out to identified the functional group and to investigate the possible roles of biomolecules. The spectra shows 18 peaks, out of which 3389.01 cm^{-1} showing N-H-1^0,2^0,amines,amides,2924.13 cm^{-1}, C-H stretching-alkenes, 1411.28 cm^{-1} C-C and C-H bend- aromatic and alkenes. (Shib shankar,2013)

CHNS analysis of **Bark** methanolic fraction reveals the presence of percentage of Carbon (38.581%), Hydrogen (7.391)%,Nitrogen (3.263%). (Anoop Singh 2010).

Table 3: FRIR Spectra Of Bark Methanol Fraction showing Functional Group

Peak No.	Peak Range	Functional Groups
2	3389.01	N-H-1^0,2^0, amines, amides
3	2924.13	C-H stretching- alkanes
8	1411.28	C-C and C-H bend -aromatics and alkanes

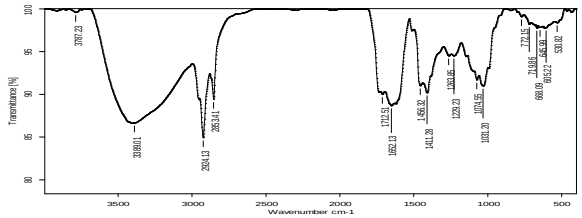

Figure 18: FTIR spectra Of Bark Methanolic Fraction

The **LC-MS** spectra of **Bark** methanol fraction shows 29 peaks and from spectrum 1A with maximum Base peak 149.1 (792482 = 100%) 12.726min, Scan: 764, 100: 1000, Ion:1659 us, RIC:1.098e+7. And in spectrum 1B with maximum Base peak: 457.4 (1.913e+6=100%) 16.916 min, Scan: 1023, 100: 1000, ion: 1450 us, RIC: 1.472e+7. (Yun Jeong Hong, Tomas-Barberan, 2006)

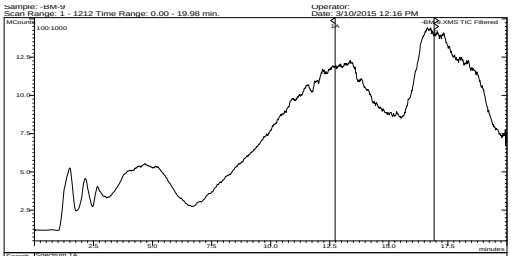

Fig 19: LC-MS spectra of Bark Methanol Fraction

The **GCMS** spectra of **Bark** methanolic fraction showed 30 peaks indicating the presence of thirty compounds, out of which 7 major compounds described and identified at Rt.39.71 minutes includes: Vitamin E (probability-49.98), Lup-20(29)-en-3-ol, acetate; (3α) (probability- 58.69) , α-Amyrin (probability-24.38), 12-Oleanen-3yl acetate (probability-26.11), 4, 4, 6a, 6b, 8a, 11,12, 14b-Octamethyl- 1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b- Octadecahydro- 2H- picen- 3-one (probability-46) (Margareth et al, 2009).

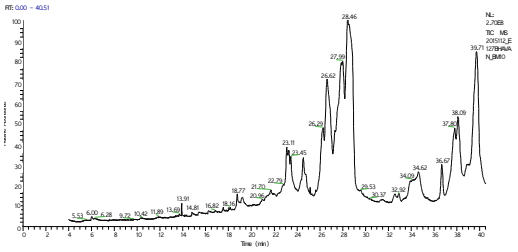

Fig 20: GCMS spectra of Bark Methanol Fraction

Table 4: Table showing peak values of LC-MS spectra of Bark Methanol Fraction

Peak No.	Base peak 149.1 (792482 = 100%) 12.726min, Scan: 764, 100: 1000, Ion:1659 us, RIC:1.098e+7	Spectrum 1A		Peak No.	RI 100 Base peak: 457.4 C: : (1.913e+6=100%) 1.4 100 16.916 min, Scan:	Spectrum 1B
1		120.8		1		149.2
2		**149.1**				
3		184.2		2		226.5
4		279.2		3		309.6
5		359.8		4		310.7
6		391.6		5		383.7
7		429.9				
8		457.3		6		**457.4**
9		514.7		7		458.1
10		586.1		8		459.4

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11		705.5		9		541.9
12		726		10		633.7
13		766.7				
14		836.2		11		726.2
15		832.2		12		832.2
16		932.8		13		909.3

Table 5: Result of GCMS of Bark Methanol fraction

Peak No.	Retention Time	Name of Compound	Chemical Formula	Molecular Weight	Probability	Activity
1	13.77	Vitamin E	$C_{31}H_{52}O_2$	430	49.98	powerful antioxidant, free radical scavenger. Anti-aging. Anti-diabetic, Anti-inflammatory Anti-dermatitic,Anti-leukemic, Hepatoprotective Hypocholesterolaemic Anticarcinogenic, Vasodilator, Antispasmodic antifertility anti-stroke, anti-thrombotic, Anti-cataract
2	23.27	Lup-20(29)-en-3-ol,,acetate,(3α)	$C_{32}H_{52}O_2$	468	58.69	anti-microbial, anti-oxidant, antiprotozoal, anti-inflammatory anti-tumor and chemo preventive properties. anti-feedants
3	25.62	α-Amyrin	$C_{30}H_{50}O$	426	24.38	anti- inflammatory and antinociceptive anti-oxidant,antipruritic,gastroprotective and hepatoprotective effect, inhibited platelet aggregation anti-fungal.
4	27.09	12-Oleanen-3-yl acetate, (3α)/ α-amyrin acetate	$C_{32}H_{52}O_2$	468	26.11	analgesic, anti-diabetic, antibacterial
5	34.62	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424	46	A nutrient, surfactant, stabilizer, used in industries as source of flavour and aroma
6	37.80	Lup-20(29)-en-3-ol,,acetate,(3α)	$C_{32}H_{52}O_2$	468	23	anti-microbial, anti-oxidant, antiprotozoal, anti-inflammatory anti-tumor and chemo preventive properties. anti-feedants
7	39.71	α-Amyrin	$C_{30}H_{50}O$	426	20.09	anti- inflammatory and antinociceptive anti-oxidant,antipruritic,gastroprotective and hepatoprotective effect, inhibited platelet aggregation anti-fungal.

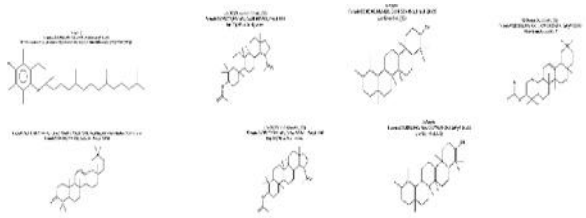

Fig 21: The structure of main compounds identified by GC-MS

On the basis of HPTLC, CHNS, FTIR, LC-MS, GC-MS analysis it may be inferred that **Lup-20(29)-en-3-ol, acetate; (3α) ($C_{32}H_{52}O_2$) with probability-58.69%** is the bioactive compound present in the bark methanol fraction belonging to pentacyclic triterpenes having the chemical formula $C_{32}H_{52}O_2$ non-polar in nature showing a wide range of antimicrobial, antioxidant, antiprotozoal, anti-inflammatory, anti-tumour, anti-feedant and chemo preventive properties. (2,4,8,9,17,18)

The present study reported that **Antioxidant Levels of** Phoenix dactylifera might have the ability to supress the free radicals. The Bark, methanol fraction (12.078mg/ml) showed good antioxidant levels. (Guo et al, Vennilla 2014)

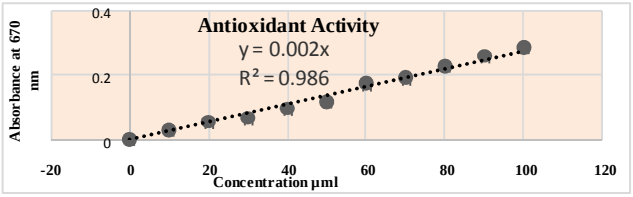

Fig 22: Graphical representation of Antioxidant activity of Bark Methanol Fraction

The bark methanol fraction of Phoenix dactylifera showed antiviral activity with an **MIC** of 780 µg/ml for the coli phage tested. Then the nature of inhibition was evaluated by performing kinetics of the bark fraction extract on the phage infectivity. (21) In the earlier studies Sabah A. A. Jassim, 2010 acetone extract from pits of Phoenix dactylifera L date demonstrated antiviral activity with an MIC value of <10 µg ml^{-1} for the Pseudomonas phage ATCC 14209-B1.

Fig 23: MIC result of antiviral activity of Bark methanol fraction

The methanol bark fraction is 99.29% capable to inhibit infectivity of coli phage and completely prevents bacterial lysis at 30 minutes incubation which shows its potential as a novel antiviral agent that can promote research against pathogenic human viruses.

Table 6: Percentage reduction of bark methanol fraction

Serial no	Sample fraction	No: of pfu/ml		% Reduction
		Control	Test	
1	Bark (Ethyl acetate)	71.16×10^7	1.3×10^7	98.17%
2	Bark (Methanol)	71.16×10^7	0.5×10^7	99.29%

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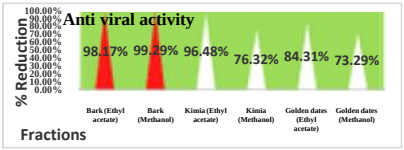

Fig24: Graphical representation of antiviral activity of bark methanol fraction

Based on the maximum inactivation of phage by bark methanol fraction leading to the evaluation of phage inhibition kinetics. The effect of bark fraction on phage's life cycle shows 13% inhibition after 1 minute exposure, while approximately 70% at 20 min of exposure. This is the probability that the one life cycle of coli phage completes approximately 20 minutes indicating the effect of fraction may be on release of from the host. (11**)**

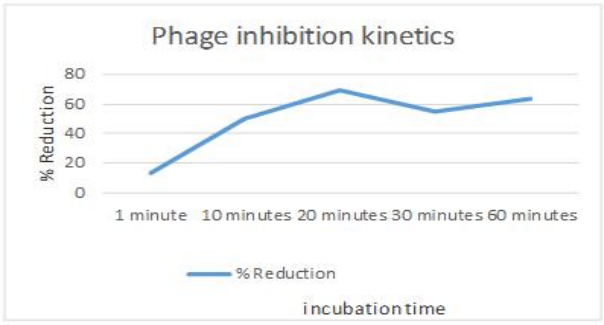

Fig25: Graphical representation of Phage Inhibition Kinetics of bark methanol fraction

The bark methanol fraction has shown an excellent antibacterial activity against **MDR** human pathogens which is in the range of 12mm-14mm revealing the potential for wide variety of infection so thereby this date bark may be considered as defence tool against array of microbes and may be have the capacity to replace traditional medicines. An extensive study would be needed to extrapolate laboratory results into hospital settings for the benefit of mankind. (Dubey and Padhy, 2013, Sahu et al., 2015).

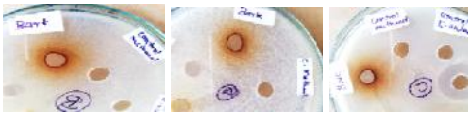

Fig 26: Image showing MDR pathogens against Bark methanol fraction by agar cup method

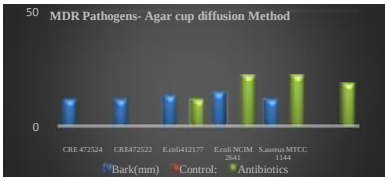

Fig 27: Graphical representation of MDR pathogen Analysis by agar cup method using Bark fraction

Table 7: Result of MDR pathogen analysis by agar cup method using Bark fraction

Sl No	Test cultures	Bark (mm)	Control: Methanol (mm)	Antibiotics-Streptomycin(mm)
1	CRE 472524 **(A)**	12	---	---
2	CRE472522 **(B)**	12	---	12
3	E.coli 412177 **(C)**	13.5	---	22
4	E.coli NCIM 2641	14	---	22
5	S.aureus MTCC 11144	12	---	18

4. CONCLUSION

In recent years, an explosion of interest in the numerous health benefits of dates has led to many studies , identification and quantification of various classes of phytochemicals with a great potential uses in the booming industries of functional foods and nutraceuticals. Researchers have found that phytochemicals have the potential to stimulate the immune sustem, prevent toxic substances in the diet from becoming carcinogenic, resuce inflammation, prevent DNA damage and aid DNA repair, reduce oxidative damage to cells, slow the growth rate of cancer cells, trigger damaged cells to self- destruct (apoptosis) before they can reproduce, help regulate intracellular signiling of hormones and gene expression, and activate insulin receptors. The study has revealed the presence of phytochemical constituent like Tannins, Saponins, Flavonoids, Glycosides, Alkaloids and Terpenoids. Out of these presence of high levels of compounds from Terpenoids family indicates Antimicrobial, antiviral and antioxidant potential. The bark fraction also display high levels of antibacterial activity against MDR pathogens. The presence of Vitamin E which is a fat-soluble strong

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antioxidant was also detected. It is expected that the antiviral property of bark methanol fraction will be of great significance in further refinement of antiviral drug design and development as potential bio therapeutic agents against medically important pathogenic human viruses, such as the human immune deficiency virus (HIV). As such the findings elucidated in the present study are expected to find practical application in diverse fields such as pharmaceuticals, food processing, nutraceuticals, Ayurveda, cosmetics, biotechnology, fisheries, nanomedicine, agriculture, bio pesticide, green chemistry, phytomedicinal research etc. We fervently hope that this study will contribute in a small but significant way to the ever expanding realm of knowledge and research in the field of Microbiology and Phytomedicinal research.

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