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Preliminary phytochemical screening, quantitative analysis, of antioxidant and antibacterial activity of crude plant extracts from *Citrus Sinensis l.*

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Abstract
The present study of Consecutive describes ofn- Hexane, Petroleum ether, Ethyl acetate, Acetone, Methanol, plant leaf extract of the *Citrus sinensis L.* for investigated for the preliminary phytochemical analysis, the polar and non-polar solvent was screened for the presence of alkaloids, Flavonoids, saponins, glycosides, anthraquinone, phenol, tannins. Antioxidant activity DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. $ABTS^+$ radical scavenging activity $ABTS^+$ (2, 2′ Azinobis (3-ethyl benzo-thizoline-6-sulfonic acid. Antimicrobial activity different solvent in highest inhibition zone for methanol extract the microorganisms for (gram – positive) *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Bacillus megaterium, Corynebacteriumdiphtheria*. (Gram-negative) *Klebsiella pnemonia, Escherichia coli, pseudomonas aeruginosa, proteusvolgaris, pseudomonas fluorescens, pseudomonas veronii. Citrus sinensis L.* Methanol were found be most effective showing the largest zone inhibition against. Further studies indicated that the gas chromatography compound analysis to the fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC – MS.

Keywords: *citrus sinensis l*, phytochemical, antibacterial, antioxidant, GC–MS analysis

Introduction
Medicinal plants are of great importance to the health of in individuals and communities in general. The medicinal value of plants lies in some chemical substance that produce a definite physiological action on the human body the most importance of these bioactive constituents of plant phytochemical contents for and number of compound present so many of the medicinal plants used for herbal medicine in world level importance plants in India [1]. Antioxidant activity of compounds and natural extracts has been recognized by that which of these natural antioxidants are more efficient, has been taken into account. Free radical produced in biological system head to cellular damages, and consequently cell death antioxidants are constituents which effectively prevent oxidation or reduce its speed through curbing free radicals in different ways [2]. Antioxidants are substances able to prevent or retard the oxidation caused by active oxygen species at low concentration [3]. Nowadays, utilization of synthetic antioxidants in medicine agriculture and pharmaceutical industry is so prevalent however many studies have shown their toxicity 36 the advent of science and technology started when humans stroke the first of light. From a nomadic life they started to colonies in civilization. Medical research and health sector of developments have always been at zenith of importance medicinal plants and natural products in amelioration of not toxicity [4]. Therefore investigation of antioxidants are focused on naturally – occurring substance especially plant phytochemicals, for increasing the Plant contain a diverse complement of metabolites [5]. In addition, the use of herbal medicine for the treatment of diseases and infection is as old as mankind. The world health organization supports the use traditional medicine provided they or proven to be efficacious and safe. This can be due to easy accessibility and availability of leaves among other plant parts preferred by most of the Ethobotanical studies. Because in is less likely to affect the Several of plant [6]. Microbial drug resistance is creating severe problems worldwide. Medicinal important plants are rich source of phytochemicals these active compound have antimicrobial properties over a period of years, various plant based active compound [7]. In recent times, focus on plant research has improved all over the world and increasing number of studies have demonstrated potential health benefits of the medicinal plant used in numerous traditional system. It has been shown that many parts of plant [8]. Search for bioactive compounds from plants with alternative mechanisms of action to counteract pathogenic microbes and natural antioxidants capable of protecting the body against oxidative stress free radical –induced damage [9]. The plant kingdom harbors enormous amounts of therapeutic agents that have diverse applications in the pharmaceutical nutraceuticals and agrochemical industries the active principles responsible for therapeutic effects of medicinal plants of phytochemicals, usually secondary metabolites including metabolites [10]. The reported 50, 0000 species of higher plants, only about 7 and 18% have been evaluated for biological activity and phytochemical analysis respectively [11]. According to the (WHO) world health organization for over 50% of report, diabetes is set to elevate from 171 million to 366 million worldwide by the year [12]. Euphorbiaceae family plant species is a small erect or ascending annual herb reaching up to 50 cm, with hairy stems it is known as plant in traditional Indian medicinal systems leaves of Euphorbiaceae plant used in the treatment of curia, cough, asthma bronchial infections, bowel complaints, hermitic infections, wounds, kidney stone and abscesses [13]. The popularly known as false daisy is a well-known medicinal plant which has been used in conventional systems of medicinal and also by traditional healers, particularly china in India as a treatment of various diseases

[14]. Worldwide, there is a belief that herbal medicines are safer to medicine used than synthetic drugs. The world health organization indicates 80% medicine, which involves the use of plant extract or their active constituents [15]. The investigation of sitling reserve forest malayali tribal peoples on the medicinal plant information gathering in to plant *Citrus sinensis L*. medicinal plants. The sitling village regions are situated on high altitude and have to environmental condition nice. In the present study, the knowledge the detailed studies on is information on the bioactivity and the phytochemical composition of this plant. There for in this present study of phytochemical screening plant and antimicrobial activity gram positive and gram negative bacteria of *Citrus sinensis L* this is the plant for first reported to the against bacterial activity and the antioxidant properties of the free radical scavenging and GC-MS analysis.

Materials and Methods

Sample collection

Sample of *Citrus sinensis L*. (Rutaceae) leaf collected from sitlingvillage Dharmapuri District, Tamil Nadu, India. *Citrus sinensis L*. leaf collected month November to December these collected samples were authenticated in (BSI) Botanical survey of India. Tamil Nadu (Coimbatore) Voucher specimens were deposited in herbarium Voucher No: BSI/SRC/5/23/18/Tech/ 2238.

Sample preparation and Extraction

The fresh plant *Citrus sinensis* ***L.*** leaf collected in the cleaning for running tap water in the plant material after then plant dried for fourteen days room temperature. The different solvent leaf was extracted in hexane, chloroform, ethyl acetate, acetone, methanol for each solvent. The solvent used for the extraction procedure in the present study were solvent (at 5ml/100ml) about 50g of dried leaf powder was extracted using 350ml of the solvent extraction with continue for the soxhlate extraction method. The filtrates for whatman paper were concentrated using aevaporator 45^0c and sample stored at 4^0c in air tight containers for further research test use.

Phytochemical screening

Citrus sinensis ***L.***prepared from each of sample or solvent n-hexane, Ethyl acetate, petroleum ether, Acetone, methanol different standardized the procedures [16].

Test for Alkaloids

0.5 g of the extract was stirred with 5ml of the 1% sample hydro choric acid on a few drops of Dragendroffs reagent were used to treat 1ml of the filtrate. The precipitation with this presence of alkaloids.

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small quantity of the extracts of the plant sample. Expeditious for development of the red colour was taken as indication of the presence of Flavonoids.

Test for Saponins

0.5g of the extract was dissolved in distilled water 2ml than agitated severely for 30 s foam stability for half an hour is the sing of the presence of saponins.

Test for Glycosides

The extract was dissolved in acetic acid glacial subsequently some drops of ferric chloride 5% and concentrated sulfuric acid were added to it. Development at brown and red colour at the inter face of the layers demonstrate the entrance of glycosides.

Test for Anthraquinone

0.5 g of the *Citrus sinensis* ***L.*** extract was boiled with 5ml of sulfuric acid 10% H_2SO_4 and filtered. Development of the pink, red or violet colour in the ammonia phase represents a positive result.

Test for Tannins

0.20 g of extract was dissolved in 10 ml of distilled water. Than 5ml of NaCl 10% was added to it two drops of chloroferic 5% were subsequently added to the extract solution. Development of a green colour reveals the occurrence of tannins.

Test for Phenol

Few drops of 5% glacial acetic acid were added to 1ml of plant extract samples followed by addition of few drops of $NaNO_2$ solution muddy brown colour formation revealed the existence of phenol present.

Quantitative analysis of phytochemicals, Estimation of total alkaloids, Estimation of total phenol, Estimation of total flavonoid, Estimation of tannin content

Estimation of total alkaloids (Harborne, 1973)

10 mg of plant material was homogenized in a mortar and pestle. Added around 20ml of methanol: ammonia (68:2). Decanted the ammoniacal solution and after 24 hrs and added fresh methanolic ammonia. Repeated the procedure thrice and pooled the extracts. The extracts were evaporated using a flash evaporator. Treated the residue with 1N HCl and kept it overnight. Extracted the acidic solution with 20ml of $CHCl_3$ thrice, pooled the organic layers and evaporated to dryness, basic fraction. Bascified the acidic layer with conc. Sodium hydroxide to pH-12 and extracted with $CHCl_3$ (20ml) thrice, pooled the $CHC1_3$ layers, dry over absorbent cotton and evaporated to dryness. Weighed the fraction that contains ajmalicine and the serpentine expressed as mg/g. [17]

Estimation of total phenol (Singleton and Rossi, 1965)

Phenols react with phosphomolybdic acid of Folin-Ciocalteau reagent in alkalinemedium and produce a blue colored complex (molybdenum blue) that can be estimated calorimetrically at 650 nm.20% Sodium carbonate, Folin-Ciocalteaureagent Standard gallic acid: 10 mg of gallic acid/10 ml of distilled water, 80% methanol. 0.5 g of sample was weighed and ground with a mortar and pestle in 5 ml of ethanol. It was centrifuged at 2000 rpm for 10 min and the supernatant was collected in 50 ml volumetric flask. Then the residue was evaporated which is dissolved in a known volume of water and used for the assay. An aliquot of the sample extract (0.1 ml) was mixed with distilled water (3 ml) and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath exactly 1 min. It was then cooled and absorbance was measured at 650 nm using spectrophotometer against the reagent blank. A calibration curve was constructed with

different concentration of gallic acid (0.01-0.1 mM) as standard was prepared and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract [18].

Estimation of total flavonoid (Ordonez *et al*., 2006)

Flavonoids react with aluminum chloride in Methanol solution forms a yellow color which was read calorimetrically at 420 nm.2% Aluminum chloride, Methanol, Stock standard, 10 mg of quercetin/10 ml distilled water. A volume of 0.5 ml of 2% of AlCl3 in ethanol solution was added to 0.5 ml of the sample solution. After one-hour incubation at room temperature, the yellow color was developed. This was measured at 420 nm with UV-Visible spectrophotometer. A standard graph was prepared using the quercetin and the total flavonoid content was expressed as quercetin equivalent (mg/g) [19].

Estimation of tannin content (Schenderl, 1970)

Tannins are widespread in nature and probably in all plant materials. The polyphenolic compounds are divided into 2 main groups, hydrolyzable and condensed. The estimation of tannin is based on the stoichiometric oxidation of molecules containing a phenolic hydroxyl group. Tannin reduces phosphor molybdic acid in alkaline condition to lower oxides of molybdenum producing a color complex, the absorbance of which is measured at 700 nm.Folin-Denis reagent: 100 g sodium tungstate and 20 g phosphomolybdic acid were dissolved in 750 ml distilled water in a suitable flask and 50 ml phosphoric acid was added. The mixture was refluxed and was made for 2 hours and makes up to 1L with water and stored in the brown bottle.35% Na2CO3: The solution was allowed to stand for overnight, filtered throughglass wool and used.Standard tannic acid solution (0.1 g/dl).0.5 g of powdered material was weighed and transferred to a 250 ml conical flask and then 75 ml water was added. The flask was heated gently for 30 min. Then it was centrifuged at 2000 rpm for 20 min and the supernatant was collected in a volumetric flask and made up to the mark.0.2-1.0 ml of standard tannic acid solution was pipetted out into a series of test tubes. To another test tube, 0.5 ml of extract solution was taken. The volumes of all the tubes were made up to 3 ml with distilled water. 3 ml of distilled water was taken as blank. To all the tubes added 5 ml of 35% Na2CO3 followed by the addition of 2.5 ml of Folin-Denis reagent and incubated at room temperature for 30 minutes. The absorbance was read against reagent blank at 700 nm. From the standard graph the amount of tannin present in the sample was calculated [20].

Antioxidant DPPH radical scavenging activity

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm. Reagents 0.2mM DPPH, 80% Methanol, Butylated Hydroxyl Anisole [17]

$ABTS^+$ radical scavenging activity

$ABTS^+$ decolourisation assay involves the generation of the *Citrus sinensis* ***L.*** plant $ABTS^+$ chromophore by the oxidation of $ABTS^+$ with potassium persulphate. It is the Applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the *Citrus sinensis L.* leaf extracts on $ABTS^+$ radical cat ion was measured at 734 nm [18]

Antibacterial assay

The antimicrobial activity crude extract for disc diffusion method [19]. The followed for estimating the antimicrobial activity of *Citrus sinensis* ***L.*** leaf extract Petriplates were prepared the (MHA) Mueller Hinton Agar were swabbed test culture were the assessed against gram positive and gram negative bacteria and the different concentration of were prepared in the suitable solvent. Next than resulting solution were than sterilized the blank disc were immersed extract solution for 20min so that they completed smeared, *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Bacillus megaterium, Corynebacterium diphtheria*. (G^+ tive) *Klebsiella pnemonia, E.coli, pseudomonas aeruginosa, proteusvolgaris, pseudomona, fluorescens, pseudomonas veroni.* The plates were incubated for 24 hours at 37^0c of inhibition was recorded.

Results and Discussion

Consecutive describes of Hexane, Petroleum ether, Ethyl acetate, Acetone, Methanol, plant leaf extract of the *Citrus sinensis* ***L.*** for investigated for the preliminary phytochemical analysis, the polar and non-polar solvent was screened for the presence of alkaloids, Flavonoids, saponins, glycosides, anthraquinone, phenol, tannins. The phytochemical screening as seen from (Table:

Table 1: Phytochemical analysis of different solvent *Citrus sinensis L*

Phytochemical test	Hexane	Ethyl acetate	Petroleum ether	Acetone	Methanol
Alkaloids	+	-	+	+	+
Flavonoids	-	-	+	-	+
Saponins	+	+	-	+	+
Glycosides	-	-	+	-	+
Anthraquinone	-	+	-	+	+
Tannins	+	-	-	-	+
Phenol	-	-	+	+	+

Key note: +, indicates Present of phytochemicals; –, indicates absent of phytochemical.

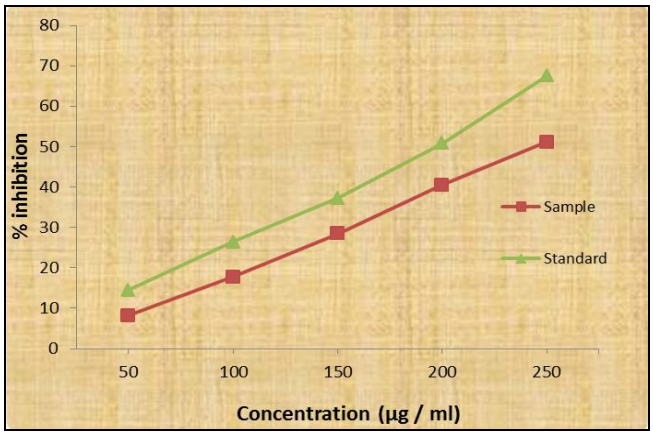
Citrus sinensis ***L.*** plant leaf for DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by it's the decrease in absorbance at wavelength 517 nm. Reagents 0.2mM DPPH, 80% Methanol, Butylated Hydroxyl Anisole. Various concentrations of the sample (4.0 ml) were mixed with 1.0 ml of solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixtures were the shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control.

The percentage of inhibition in DPPH free radical scavenging activity was calculated as follows; As seen from (Table:2), (fig:1)

Table 2: Antioxidant activity of Methanol Extract *Citrus sinensis L* DPPH radical scavenging activity.

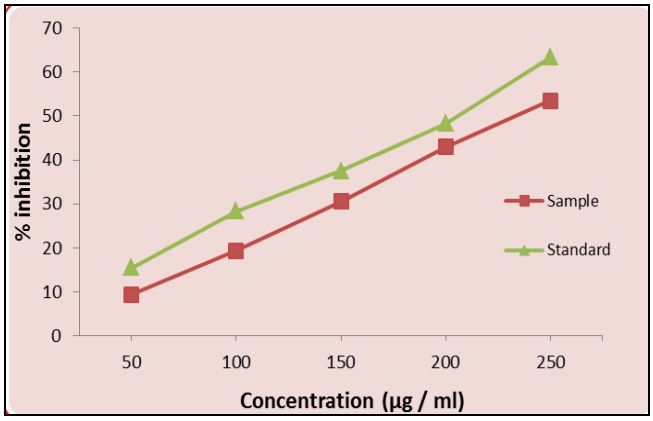
(MET)	Radical scavenging assay Methanol Extraction different concentration % inhibition				
	50(µg/ml)	100(µg/ml)	150 (µg/ml)	200(µg/ml)	250 (µg/ml)
Sample	8.30 ± 0.50	17.70 ± 0.78	28.50 ± 0.10	40.62 ± 0.80	51.26 ± 0.90
Standard (Vitamin C)	14.50 ± 0.89	26.40 ± 0.10	37.28 ± 0.90	50.80 ± 0.10	67.50 ± 0.30

1. MET: methanol extract
2. Each value is represented as mean ± SEM (n = 3)



IC_{50} value of Sample: 244.08 µg/ml
IC_{50} value of Vitamin C (standard): 197.40µg/ml

Fig 1: The percentage of the free radical DPPH by the antioxidant Vitamin C. *Citrus sinensis*



IC_{50} value of Sample: 232.75 µg/ml
IC_{50} value of Vitamin C (standard): 206.68 µg/ml
% Inhibition = A0- A1/A0 X 100

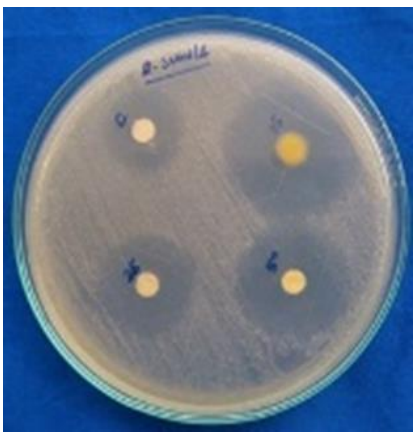
Fig 2: *Citrussinensis* $ABTS^+$ radical scavenging assay

$ABTS^+$ de colorization assay involves the generation of the $ABTS^+$ chromospheres by the oxidation of $ABTS^+$ with in potassium persulphate. It is applicable for the both hydrophilic and lipophilic compounds. The scavenging activity of the *Citrus sinensis* ***L.***leaf extracts on $ABTS^+$ radical cation was measured at 734 nm. Reagents 7 mM $ABTS^+$(2, 2′ Azinobis (3-ethyl benzo-thizoline-6-sulfonic acid), 2.45 mM Potassium persulphate, $ABTS^+$ solution: Equal volume of 7 mM of $ABTS^+$ was mixed with 2.45 mM potassium persulphate and the mixture was allowed to the stand in the dark at the room temperature for 12-16 hours before use. $ABTS^+$ solution was diluted to an absorbance of 0.7 ± 0.05 with ethanol at 734 nm. Ascorbic acid. The reaction was initiated by the addition of 1.0 ml of diluted was $ABTS^+$ to 10 µl of different concentrations of (5-100 µg / ml) of sample and also to 10 µl of ethanol as a control. Ascorbic acid was used as positive control. The absorbance was read at 734 (nm) after 6 than minutes and the percentage inhibitions were calculated. The inhibition was calculated according to the equation, as seen from (Table: 3), (fig: 2)

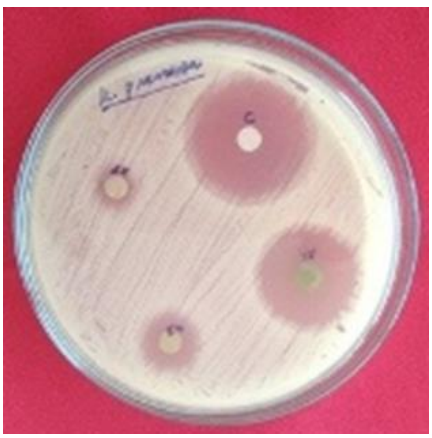
Table 3: Antioxidant activity of Methanol Extract *Citrus sinensis L*$ABTS^+$ radical scavenging activity

(MET)	Methanol Extraction different concentration % inhibition				
	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)
Sample	9.43± 0.10	19.42 ± 0.20	30.69 ± 0.40	42.94 ± 0.40	53.50 ± 0.50
Standard (Vitamin C)	15.50 ± 0.65	28.38 ± 0.50	37.50 ± 0.20	48.20 ± 0.40	63.38± 0.20

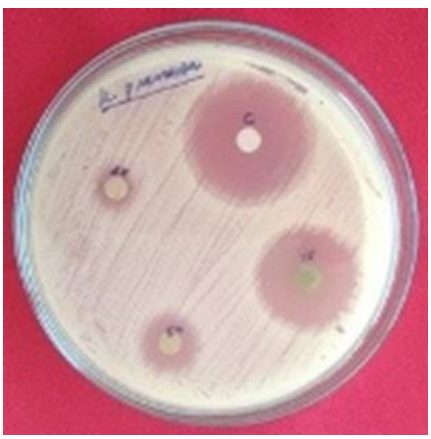
MET: methanol extract
Each value is represented as mean ± SEM (n = 3)
$I = A_o – A_1/A_o$ x 100,



B. subtilis



K. Pneumonia



S. aureus

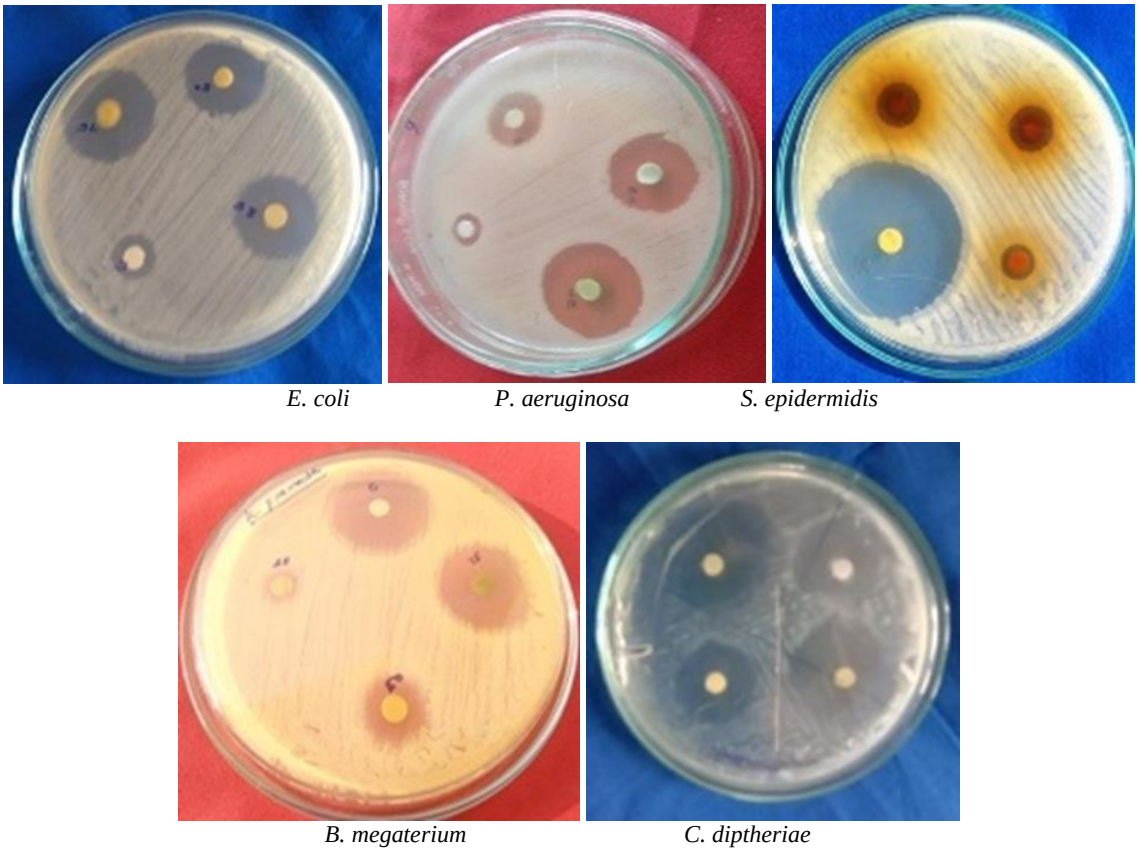


Fig 3: Antimicrobial activity *C. sinensis L.* Antimicrobial activity *Citrus sinensis* in methanol extract gram positive and gram Negativ Bacteria

Table 4: Antibacterial activity of Methanol extract of *Euphorbia notoptera* against gram positive and gram negative bacteria.

organisms	(25µ) Concentration	(50 µ) Concentration	(75 µ) Concentration	P. control Sterptomycine
Bacillus subtilis	6.33 ± 0.33	8.00 ± 0.57	9.66 ± 0.33	13.00 ± 0.57
Klebsiella pneumonia	9.66 ± 0.33	12.00 ± 0.57	15.00 ± 0.57	22.66 ± 0.88
Staphylococcus aureus	7.66 ± 0.88	11.00 ± 0.57	14.66 ± 0.88	17.66 ± 0.66
E. coli	10.00 ± 0.57	13.66 ± 1.20	17.00 ± 2.30	24.00 ± 1.52
Pseudomonas aeruginosa	10.00 ± 0.57	12.33 ± 0.33	14.00 ± 0.57	18.00 ± 0.57
Proteus vulgarise	-	-	-	16.66 ± 1.45
Staphylococcus epidermidis	6.66 ± 1.20	10.66 ± 0.88	15.00 ±0.88	16.66 ± 1.85
Pseudomonas fluoresces	6.33 ± 1.20	10.00 ± 0.57	14.33 ± 0.88	18.66 ± 0.88
Staphylococcus pneumonia	10.33 ± 0.88	12.66 ± 1.45	16.66 ± 1.20	22.00 ± 1.52
Entrococeusfaecalis	-	-	-	15.33 ± 1.76
Pseudomonas veronii	7.00 ± 1.15	10.66 ± 1.45	15.33 ± 1.20	15.33 ± 1.45
Bacillus megaterium	6.33 ± 0.88	11.66 ± 0.88	16.33 ± 1.76	18.00 ± 1.00
Corynebacterium diphtheria	5.33 ± 0.88	7.66 ± 0.66	11.33 ± 0.88	12.33 ± 0.66

-, represents no activity. Values are the mean of triplicate value ± SE. Statistical analysis for found the significant at $p < 0.05$. Zone inhibition. The antibiotic disk in streptomycin for bacteria

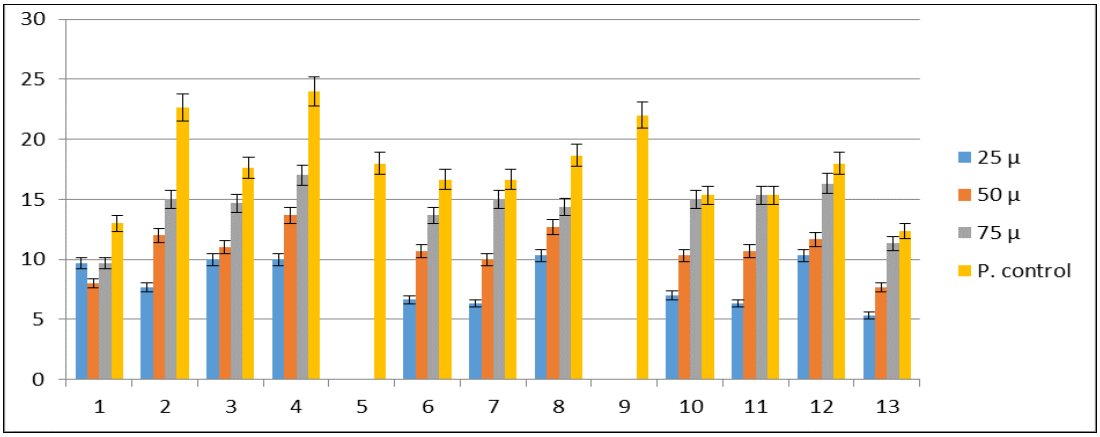


Fig 4: Antimicrobial activity *Citrus sinensis* Methanol Extract

Standard Devotion and Standard Error

Where, the A_o is absorbance of control reaction, A_1 is absorbance of test compound.

Antimicrobial activity different solvent in highest inhibition zone for methanol extract the microorganisms for highest inhibitation zone (gram – positive) *Bacillus subtilis,* (9.66 ± 0.33) *Staphylococcus aureus,* (14.66 ± 0.88) *Staphylococcus epidermidis,* (15.00 ±0.88) *Streptococcus pneumonia,* (16.66 ± 1.20) *Bacillus megaterium,* (16.33 ± 1.76) *Corynebacterium diphtheria.*(11.33 ± 0.88) (Gram-negative) *Klebsiella pnemonia,* (15.00 ± 0.57)*Escherichia coli,* (17.00 ± 2.30) *pseudomonas aeruginosa,*(14.00 ± 0.57) *pseudomonas fluorescens,* (14.33 ± 0.88)*pseudomonas veronii.* (15.33 ± 1.20) (Table:4), (fig:3) GC-MS information make Perkin Elmer model Clarus 680 mass spectrometer Clarus 600 (El) software turbo mass ver 5.4.2 Library vear NIST- 2008. The acquisition parameters oven initial temp 60^0c for 2 min, ramp 10^0c / min to 300^0c, hold - 6 min. Total run time 32. 00 mint InjAauto = 260^0c, volume = 1µL, Split = 10:1 Flow Rate: 1ML / mint. Carrier Gas = He Column = elite – 5 MS (30.0 m, 0.25 mm ID, 250µ DF). Mass Condition (EI) solvent delay = 2. 00 min, Transfer temp = 240^0c, source temp = 240^0c scan: 50 to 600 Da. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite – 5 MS (5% biphenyl 95% dimethyl polysiloxane, 30 m x 0.25 mm ID x 250 µm df) and the components were separated using helium as carrier gas at a constant flow of 1 ml / min. the l ml of extract sample injected into the instrument the oven temperature was as follows: 60^0c (2 min) followed by 300^0c at the rate of 10^0c min -1; and 300^0c, where it was held for 6 min. The mass detector condition was: transfer time temperature 240^0c; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC – MS NIST (2008) library. (Ertas, A. 2015)Fig: 5, 6: Table: 5, 6 Fig 5 GC-MS analysis peak P. ether extract *Citrus sinensis L.* Acquisition parameters Instc Acquisition parameters Ovan: initial temperature 60^0c for 2.80 min ramp 10^0c main to 290^0c hold 6 min, injAauto = 260^0 c, volume = 0 µ, split = 10:1, carrier gas = He, solvent Delay = 2.80 min, Transfer Temperature = 230^0c, source Tem = 23^0c Scan: 40 to 600 Da, column 30.0m x 250µ

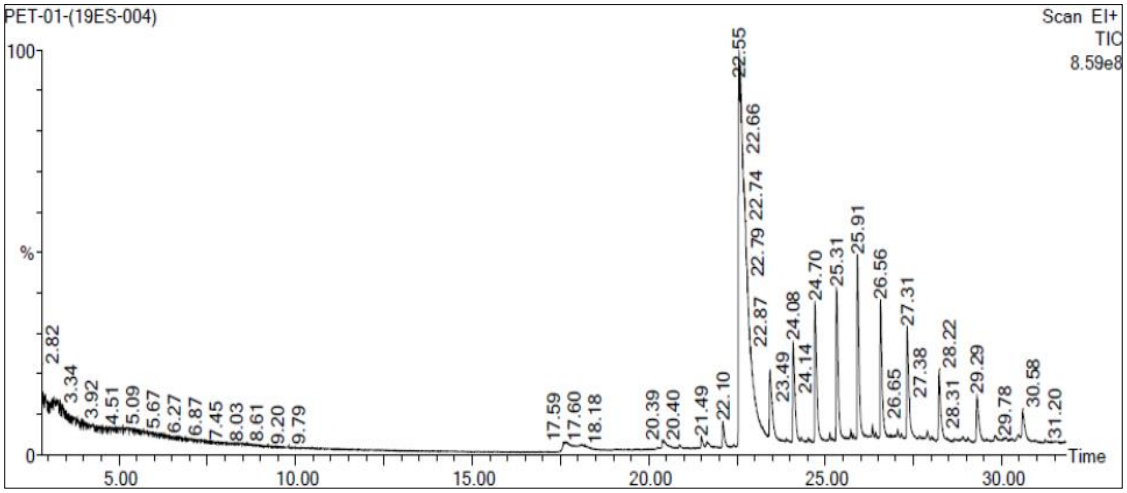


Fig 5: GC-MS analysis Petroleum Ether extract*Euphorbia notoptera*

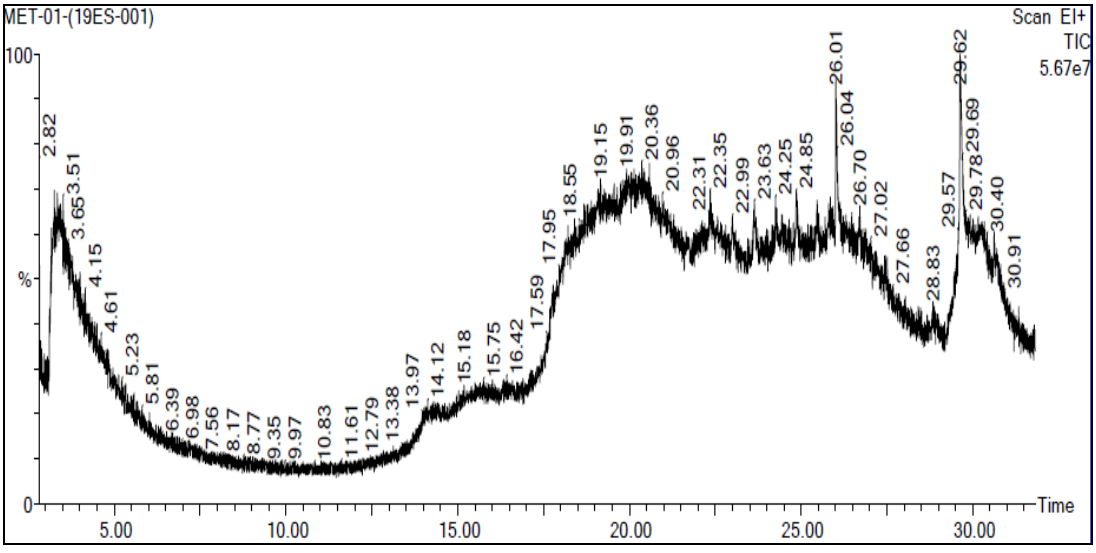


Fig 6: GC-MS analysis peak methanol extracts *Citrus sinensis L*

In this present study of Phytochemical analysis of leaf extract *Citrus sinensis* ***L.***the revealed that among the substances investigated presence of alkaloids, Flavonoids, saponins, glycosides, anthraquinone, phenol, and tannins [20]. The presence of pharmacological activities antioxidant, The percentage of the free radical DPPH by the antioxidant Vitamin c and $ABTS^+$ radical scavenging assay [21]. The result shows the values of Ic_{50} of methanol extract. The *Citrus sinensis* ***L.*** leaf and ascorbic acid as pattern, and points to a the higher antioxidant activity of this plant extract to the standard used ascorbic acid, showing the effectiveness of antioxidant activity. The antibacterial activity for high result methanol extract they showed the bacteria highest inhibition zone for methanol extract the microorganisms for (gram – positive) *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Bacillus megaterium*, *Corynebacterium diphtheria*. (Gram-negative)*Klebsiella pnemonia*, *Escherichia coli*, *pseudomonas aeruginosa*, *proteusvolgaris*, *pseudomonas fluorescens*, *pseudomonas veronii.* (*Citrus sinensis L.)* [22]. The solvent of (MET) and (PET) Petroleum ether extract Gas Chromatography Mass Spectrometry fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC – MS [23]. Name of the compound presence in (MET) O-Methylisourea. Oxiranemethanol, (S). Ala-Beta, Ala, Trimethyisilyl Ester.

2 Oxabicyclo (4.4.0) Decan -10-One, [1, 3, 7, 7]. -Tetramethyl,- (IR, 3R,6R). [1, 2] Hydrazinedicarboxamide. Phenethylamine, [2, 4, 5] Trimethoxy,-Alpha- Methyl. [3, 7]- Diacetamido – 7H – S – Triazolo [5, 1 – c] – S – Triazole. Ethanamine, 2, 2 - Oxybis.

2- Hydroxymethyl -9- [Beta, - D – Ribofuranosyl] Hypoxanthine and (PET) Di-N-octyl phthalate, Hexatriacontane, Hexatriacontane, Octacosane, Tetratetacontane, Tetracosane, Hexatriacosane, Hexatraiacontane, Heptacosane, 1 – Chloro, Sulfrous acid, 2- showed of presence of compound (GC-MS). (Library - NIST-2008)

Table 5: GC-MS Analysis name of the compound present *Euphorbia notoptera* plant leaf methanol extract

S.No	REW	Compound Name	M.W	Formula
1	723	O- Methylisourea	74	$C_4H_8O_2$
2	818	Oxiranemethanol, (S)	74	$C_3H_6O_2$
3	699	Ala-Beta, Ala, Trimethyisilyl Ester	232	$9H_20O_3N_2Si$
4	694	2 Oxabicyclo (4.4.0) Decan -10-One, 1,3,7,7-Tetramethyl,- (IR, 3R,6R)	210	$C_{13}H_{22}O_2$
5	838	1,2- Hydrazinedicarboxamide	118	$C_2H_6O_2N_4$
6	676	Phenethylamine, 2,4,5- Trimethoxy,-Alpha- Methyl	225	$C_{12}H_{19}O_3N$
7	800	3,7- Diacetamido – 7H – S – Triazolo [5,1 – c] – S – Triazole	223	$C_7H_9O_2N_7$
8	675	Ethanamine, 2, 2 - Oxybis	104	$C_4H_9O_3N$
9	751	2- Hydroxymethyl -9- [Beta, - D – Ribofuranosyl] Hypoxanthine	298	$C_{11}H_{14}O_6N_4$
10	721	2,3- Anhydro – D – Galactosan	144	$C_6H_8O_4$
11	711	Bicylo [3, 2.1] Oct – 3 – En – 2 One, 3,8 – Methoxy – 7- (-7 Methoxy -1, 3	388	$C_2,H_{24}O_7$
12	780	9,19- Cyololanost- 484		$C_{32}H_{52}O_3$

Table 6: GC-MS Analysis name of the compound present *Euphorbia notoptera* plant leaf Petroleum ether extract

S.No	REW	Compound Name	M.W	Formula
1	941	Di-N-octyl phthalate	390	$C_{24}H_{38}O_4$
2	955	Hexatriacontane	506	$C_{36}H_{74}$
3	963	Hexatriacontane	506	$C_{36}H_{74}$
4	978	Octacosane	338	$C_{24}H_{50}$
5	980	Tetratetacontane	618	$C_{44}H_{90}$
6	955	Tetracosane	338	$C_{24}H_{50}$
7	974	Hexatriacosane	506	$C_{36}H_{74}$
8	789	Hexatraiacontane	618	$C_{44}H_{90}$
9	774	Tetratetracontane	618	$C_{44}H_{90}$
10	928	Heptacosane, 1 – Chloro	414	$C_{27}H_{55}$
11	937	Sulfrous acid, 2-	320	$C_{17}H_36O_3S$

Statistical analysis

Citrus sinensis L. ware analyses by Experimental result. The Data represent the mean ± (SD) standard deviation for (ANOVA) statistical methods. Were determined using the Pearson correlation coefficient in considering P values < 0.05 were for valuation regarded to be the significant.

Conclusion

The present study of *Citrus sinensis L.* leaves this is the first report and different solvent extract n- hexane, Petroleum ether, Ethyl acetate, Acetone, Methanol, all extract showed Phytochemical screening showed the antibacterial activity results were also comparable to the positive control antibiotic (streptomycin) used as a standard reference. The antioxidant activities leaves showed highest activity against the crude extract from dry leaves showed the highest antioxidant activity and methanol. GC-MS analysis for number of responsible compound presented in *Citrus sinensis L.* leaf methanol extract for the number of compound presented for depend on the presence of phytochemical such as (alkaloids, Flavonoids Saponins, Glycosides, Anthraquinone, Tannins, phenol. This plant extract could serve as potential sources of antioxidant and antimicrobial agents and further research work anticancer study of present extract used for pharmaceutical use identify the compound isolation.

Abbreviations

ANOVA Analysis of Variance
G^+: Gram Positive
G-: Gram negative
MET: Methanol
PET: Petroleum ether
SD: Standard deviation
GC-MS: Gas Chromatography Mass Spectrometry
BSI: Botanical Survey of India
C. S: *Citrus sinensis L.*

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