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Comparative Evaluation of Antimicrobial and Anti-Inflammatory Activities of *Ocimum sanctum, Phyllanthus niruri* and *Cadaba fruticosa*: An *in vitro* Approach with Emphasis on Detection of their Bioactive Compounds Using GC-MS

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ABSTRACT

The current investigation was performed with the objective of finding the scientific reason for health benefit of *Occimum sanctum*, *Phyllanthus niruri* and *Cadaba fruticosa*, simultaneously evaluating the antioxidant activity of ethanol, methanol and aqueous extracts. The potent antioxidant, antimicrobial and anti-inflammatory leaf extracts can be used for producing a novel ointment formulation, functional food or neutraceutical, in combination. The qualitative analysis of antioxidant activity was performed by reducing power assay and DPPH assay and *in vitro* anti-inflammatory test was performed by the HRBC membrane stabilization method. Further, the bioactive compounds present in the alcoholic leaf extracts were detected by GC-MS analysis and their applications were noted. Comparative studies on antimicrobial and anti-inflammatory activity of the 3 samples were determined using respective standards. *Cadaba fruticosa* is an under-emphasized herb, whose potential in the medical world is yet to be explored in details. Hence, *Cadaba fruticosa* has been chosen as one of the herbal plants for comparative study with *Ocimum* sp. and *Phyllanthus* sp. Ethanolic extract of showed significant antimicrobial activity against the tested bacterial strains. Methanolic extract of *Ocimum sanctum* at 200 μg mL^{-1} depicted highest scavenging activity, 81.1%. The results also indicate that *Cadaba fruticosa* possesses significant free radical scavenging and anti-inflammatory activities *in vitro*. GC-MS analysis of the herbs revealed the presence of several bioactive compounds.

Key words: *Ocimum sanctum*, *Phyllanthus niruri*, *Cadaba fruticosa*, antimicrobial, anti-inflammatory, antioxidant, chromatography

INTRODUCTION

India has been the source of various herbal plants which gave rise to the world of Ayurveda. For years medical practitioners have used different parts of herbal plants (leaves, stems, flowers, roots, stems) to treat several diseases like malaria, respiratory diseases, skin diseases, insect bites and many more. Commonly found Indian herbal plants like *Ocimum sanctum*, also known as Tulsi or holy basil and *Phyllanthus niruri*, also known as kheezha nelli or stonebreaker, is known to possess antidiabetic, antimicrobial, antifungal, antispasmodic, analgesic effects (Prakash and Gupta, 2005; Syamasundar *et al*., 1985). *Cadaba fruticosa* is an underemphasized herbal plant with pharmacological potency. It is reported to have antidiabetic, antimicrobial, stimulant and purgative effects. The GC MS results will throw more light on their beneficial health effects.

The antioxidant and antimicrobial effects of phytochemicals present in herbal plants have accelerated the borders of Ayurveda, which has been used as medicine for several thousand years by common man. Antimicrobial activities of various phytochemicals have been investigated and the prospect of using them in the evolution of new antimicrobial drugs has also been recorded (Akinmoladun *et al*., 2007). With progress in the medical world, it has also seen the development of several mutant pathogenic microbes. The emergence of new resistant pathogen has drawn attention to the world of antibiotics and modern medicine. And modern medicine has pushed the limits of Ayurveda (Nascimento *et al*., 2000).

Gas chromatography-mass spectrophotometry can quantitatively determine compounds present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis. It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds and for the determination of such thermo chemical constants as heats of solution and vaporization, vapor pressure and activity coefficients. Mass spectrometry is the analytical technique based on the principle of measurement of mass-to-charge ration of the charged biological samples such as peptides. The mass spectrum contains various peaks which correspond to the m/z value of various types of peptides in the original sample.

Antioxidant activity is very common term associated with the analysis of antioxidants in any organic sample. 'Antioxidant capacity' essentially means the measure of antioxidants present in the sample to remove the free radicals under specific conditions. Scavenging activity is the percentage removal of free radicals by antioxidants. Lower absorbance value lower indicates lower presence of free radical and higher scavenging capacity. There are several methods to determine the antioxidant capacity, based on various principles. In the current investigation, reducing power assay and DPPH assay has been used to determine the antioxidant capacity (Benzie and Strain, 1999).

Humans and mammals respond to a variety of hostile foreign agents like pathogens, toxic chemicals or physical damage to the tissue through inflammation. The process associated with the inflammatory response are complex but important aspects which have been exploited for screening of anti- inflammatory compound the role played by Reactive Oxygen Species (ROS) (Aksoy *et al*., 2013; Laupattarakasem *et al*., 2003). Discovery of potent Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) with very low or no gastrointestinal (GI) side effects is area of significant interest (Grover *et al*., 2006). As a result of such long lasting problems associated with the synthetic non-steroidal as well as steroidal anti- inflammatory agents, there is continuous search especially from natural sources for alternative agents (Cai *et al*., 2007; Kaneria *et al*., 2007; Park *et al*., 2006). The objective of present study is to find the scientific reason for health benefit of *Occimum sanctum*, *Phyllanthus niruri* and *Cadaba fruticosa*, by evaluating the antioxidant activity of ethanol, methanol and aqueous extracts of the three herbs.

MATERIALS AND METHODS

Sample collection: *Ocimum sanctum* (Tulsi), *Cadaba fruticosa* (Viludhi) and *Phyllanthus niruri* (Kheelanelli) were collected from the nursery of VIT University, Vellore, Tamil Nadu. All the plants are cataloged and also authenticated by Dr. P. Jayaraman, Director of Plant Anatomy Research Centre (PARC), Tambram, Chennai, Tamil Nadu. The leaves were separated and grounded with mortar pastel using distilled water. The fine particles were then made to dry at room temperature,

after which it was packed and stored in fridge at 4°C for further use. The three bacterial strains; *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* were acquired from the Molecular Biology and Genetics lab of VIT University and was sub-cultured overnight in Nutrient Broth to be used for antimicrobial testing.

Chemicals and reagents: Commercial antibiotic discs (Methicillin, Chloramphenicol, Ampicillin and Vancomycin) used in the study were obtained from HiMedia Ltd., India. Phosphate buffer was prepared by mixing dibasic sodium phosphate (37.50 mL of 0.2 M) along with 62.5 mL monobasic sodium phosphate, which was diluted with water, to 100 mL of final solution. Alsever's solution was made using 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% NaCl. All chemicals and reagents used in the present study were of standard analytical grade and purchased Sigma Aldrich Ltd., India.

Preparation of aqueous and alcoholic extract: The dried leaves of the leaves were finely powdered and the 50 g of the powder was extracted with 200 mL of ethanol, methanol and distilled water, separately for 24 h in a glass conical flask at 25°C using a shaker. The grounded solution was ultra-sonicated for 10 min, at 100 amplitude and 6 pulsar units. Sonication process was incorporated the methodology to achieve the complete breakdown of rind cells and let the intracellular bioactive compounds be extracted in the solvent. This would give a better picture of the leaf extract properties being investigated. This was then centrifuged at 12000 rpm for 10 min. It was then filtered through Whatmann filter paper No. 1. The liquid extracts were given to dry inside hot air oven at 35°C for 48 h. Solidified filtrates were then scraped out using a blade which gave powdered consistency. All the extracts were stored in petri plates and air tight plastic and kept at 4°C, to be used for future experiments.

Antibacterial property assay: The antibacterial property was determined by disc diffusion method. The test organisms were inoculated in a 100 mL conical flask containing nutrient broth media and it was incubated overnight at 37°C. Streaking was done to obtain a pure culture line from the conical flask mixture. The pure bacterial colonies were taken and grown into 20 mL agar broth. Muller Hinton agar test plates were prepared for each test organisms. Agar plates for the bacterial cultures were prepared, taking 0.2 mL of the culture volume. It was then evenly spread using sterilized swab. About 6 mm diameter discs, in triplicates per sample extract (3 conc.×3 types of extracts×3 plant samples), were made using sterilized punched filter paper. Methicillin, chloramphenicol, ampicillin and vancomycin antibiotic discs were used as standard antibacterial agents for comparison of antibacterial activity of the leaf extracts from the test organisms. Each disc was dipped in the alcoholic/aqueous solutions of the extracts and was set on the plates. Then the plates containing the leaf extracts along with the test organisms were left at ambient room temperature to facilitate the diffusion of test samples and then incubated with face upwards at 37°C for overnight. The diameter of the zone of exclusion was measured using a measuring scale.

Antioxidant assays: Reducing power assay and DPPH assay was used to investigate the antioxidant activity in the current study.

Reducing power assay: The reducing power assay has been used to determine the determined by the method of Oyaizu (1988) which measured the antioxidant capacity of leaf extracts under

investigation, with respect to L-ascorbic acid (1% w/v) as the standard antioxidant compound. One milliliter of different samples of leaf extracts were mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was kept at a temperature of 50°C in hot water bath for the time duration of 20 min. After cooling the mixture at room temperature, 2.5 mL of 10% Trichloroacetic acid was added and further centrifuged at 3000 rpm for 10 min. About 2.0 mL of the supernatant was separated in a sterile test tube and mixed with 2.5 mL of distilled water and 0.5 mL of freshly prepared ferric chloride solution. The absorbance of the resultant mixture was measured at 700 nm by the spectrophotometer.

DPPH assay: The alcoholic leaf extracts were taken for DPPH assay determined by the method of Blois (1958). Three milliliter, 60 μM of ethanolic solution of DPPH was taken for each sample under the current study. About 250 μL of each, fruit rind extracts as well as L-ascorbic acid was mixed with DPPH. L-ascorbic acid was taken as standard antioxidant compound. The concentration of L-Ascorbic acid and the fruit rind extracts were kept as 1% (w/v). The solution was incubated at room temperature for 30 min followed by O.D. measurement at 517 nm. Ethanol was used to set zero point of the spectrophotometer. The following formula was used to calculate the free radical scavenging activity:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Gas Chromatography coupled Mass Spectrometry (GC-MS) analysis: The ethanol and methanol extract of the leaves were used for GC-MS sample. 0.5% (w/v) of the alcoholic sample was prepared in ethanol/methanol respectively solvent. The sample was properly ground in a mortar pastel to obtain a homogenized mixture. The homogenized mixture was further sonicated for 10 min at amplitude 100 and pulsar 6. The mixture was poured in a sterile petri dish and kept overnight to obtain a solid powdery sample of the rind. The powder sample was used for the GC-MS analysis. The GC-MS used in the present study was Perkin Elmer made with GC model as clarus 680 and mass spectrometer model as clarus 680 (EI). The instrumental acquisition parameter was as following. Oven: initial temperature 60°C for 2 min, ramp 10°C min^{-1} to 300°C, hold 6 min, total run time = 32.0 min. Carrier gas was helium and the column was Elite 5MS. The mass condition (EI) was kept as follows. Solvent delay = 2.00 min, transfer temperature = 230°C, source temperature = 230°C, scan = 50-600 Da.

***In vitro* anti-inflammatory test by HRBC membrane stabilization method:** The reaction mixture (4.5 mL) consisted of 2 mL of hypotonic saline (0.25% NaCl), 1 mL 0.15 M phosphate buffer (pH 7.4) and 1 mL of test solution (100-2000 μg mL^{-1} of final volume) in normal saline. About 0.5 mL of 10% rat RBC in normal saline was added. For control tests, 1 mL of isotonic saline was used instead of test solutions while product control tests lacked red blood cells. The mixtures were incubated at 56°C for 30 min. The tubes were cooled under running tap water for 20 min. The mixtures were centrifuged and absorbance of the supernatants read at 560 nm. Percent membrane stabilizing activity was calculated as follows:

$$\text{Percent stabilization} = 100 - \left(\frac{\text{O.D. of test} - \text{O.D. of product control}}{\text{O.D. of control}}\right) \times 100$$

$$\text{Percent cell haemolysis} = \left(\frac{\text{O.D. of test}}{\text{O.D. of control}}\right)\times 100$$

RESULTS

Antibacterial activity: The antimicrobial study was conducted by comparing 3 different concentrations of each extract. The potency of these plants (in terms of concentration) was measured by their Zones of Inhibition (ZOI) (Table 1). The 3 concentrations were -0.1 (A), 0.3 (B), 0.5 (C) in mg mL^{-1}. Water and methanol did not show any zone of inhibition against the 3 bacterial strains, whereas ethanol showed ZOI of 7, 6 and 4 mm for *E. coli*, *P. aeruginosa* and *B. subtilis*, respectively. Standard antibiotics were used for the purpose of comparison (Table 2).

Antioxidant activity

Reducing power assay: This test was conducted using plant leaf extracts (concentrations A, B and C already mentioned previously). Triplicates of each sample were assayed and their average was calculated. Ascorbic acid was used as standard -0.7372 absorbance (Table 3). Distilled water was used as the common solvent for dissolving the various alcoholic and aqueous extract (Fig. 1-6).

DPPH test: Antioxidant assay by DPPH method was also carried out using ethanol and methanol extracts only. Table 4 shows the absorbance measured at 517 nm. The concentrations were taken as 200 (D), 100 (E), 50 (F) μg mL^{-1}. Ascorbic acid was used as standard reference.

Table 1: Zone of inhibition of alcoholic and aqueous extracts shown on the 3 bacteria

	Aqueous extract			Methanol extract			Ethanol extract		
Treatment groups	*E. coli*	*Pseudo*	*Bacillus*	*E. coli*	*Pseudo*	*Bacillus*	*E. coli*	*Pseudo*	*Bacillus*
Ocimum sanctum									
A	-	5	3	7	12	15	6	13	15
B	-	5	3	8	17	18	8	15	19
C	3	6	4	11	21	22	10	22	20
Phyllanthus niruri									
A	-	-	-	10	7	9	11	8	10
B	-	-	-	15	8	17	13	8	17
C	-	-	-	20	12	22	19	13	22
Cadaba fruticosa									
A	8	8	-	10	7	6	9	7	8
B	10	11	-	12	9	6	12	7	6
C	11	11	6	15	10	8	14	9	5

Above table measure the zone of inhibition in mm

Table 2: Antibiotics showed zone of inhibition (mm)

Samples	Ampicillin	Methicillin	Vancomycin	Chloramphenicol
E. coli	15	0	20	24
Pseudomonas sp.	13	-	-	5
Bacillus sp.	10	-	14	25

Table 3: Antioxidant capacity observed at 700 nm

Samples	A	B	C
*O. sanctum*_Eth	0.8688	0.9122	0.9315
*O. sanctum*_Meth	0.8295	0.9351	0.9771
*O. sanctum*_Aq	0.9687	1.0122	1.1351
*P. niruri*_Eth	0.8288	0.9016	0.9351
*P. niruri*_Meth	0.8295	0.9351	0.9456
*P. niruri*_Aq	0.9687	1.0122	1.1350
*C. fruticosa*_Eth	0.0071	0.5506	0.9506
*C. fruticosa*_Meth	0.2215	0.8771	0.9577
*C. fruticosa*_Aq	0.0071	0.2506	1.2506

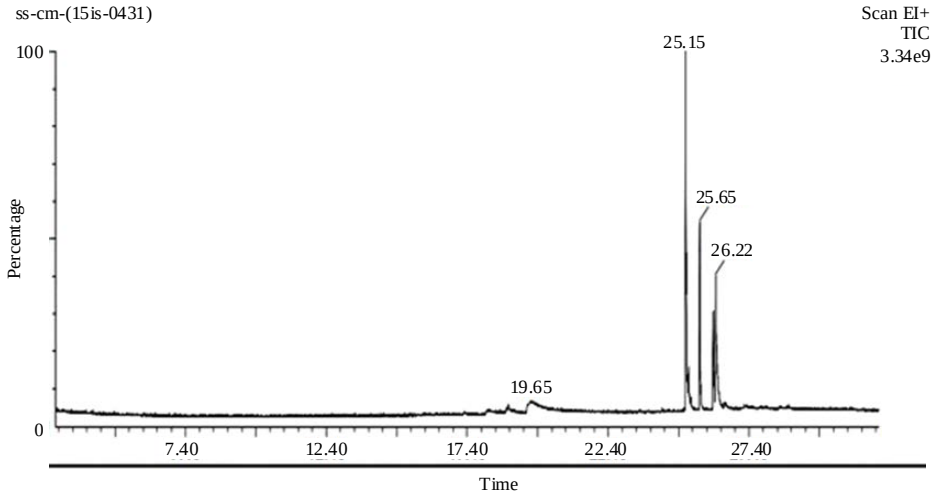

Fig. 1: Chromatogram of methanolic extract of *Ocimum sanctum*

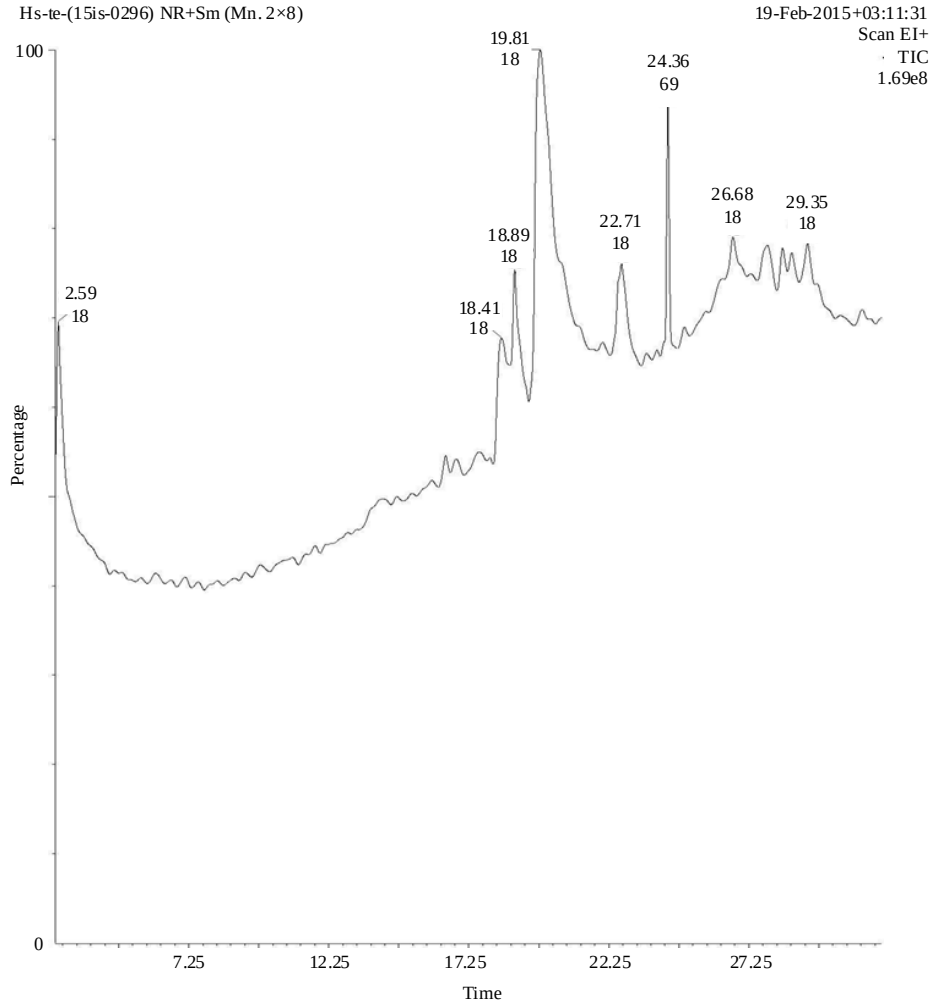

Fig. 2: Chromatogram of ethanol extract of *Ocimum sanctum*

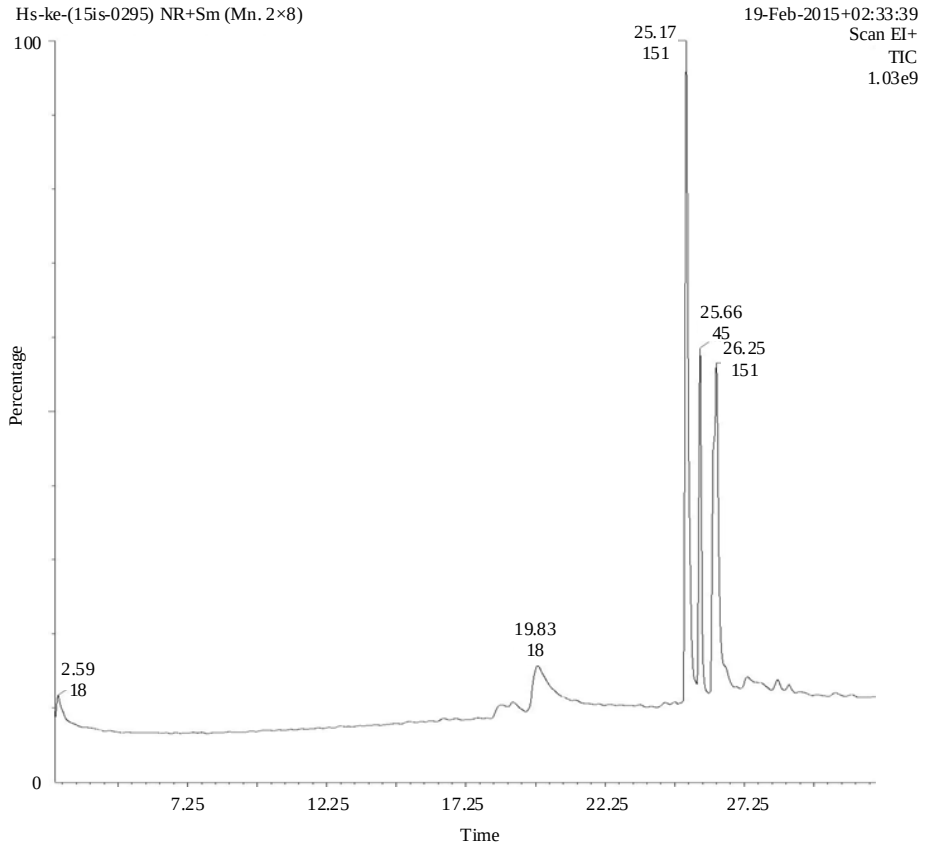

Fig. 3: Chromatogram of ethanol extract of *Phyllanthus niruri*

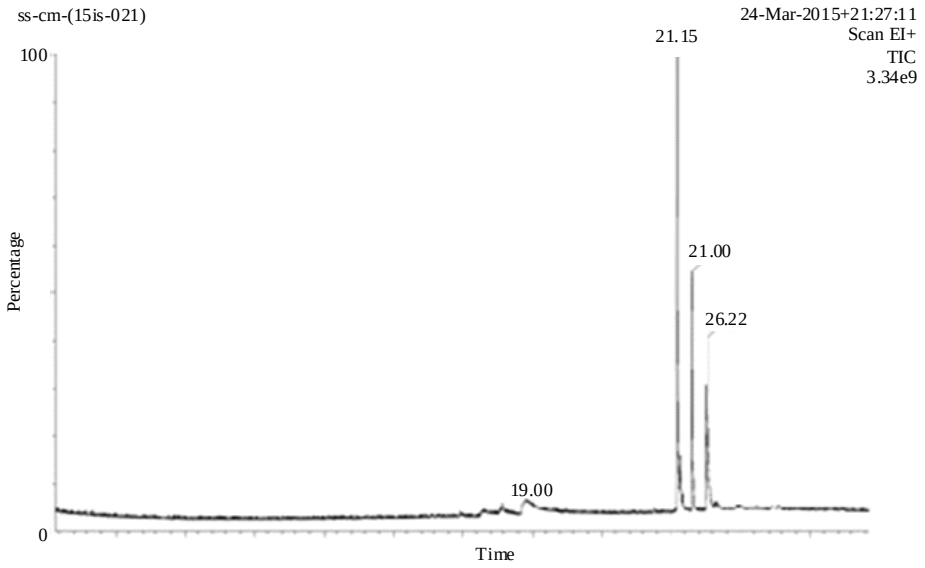

Fig. 4: Chromatogram of methanol extract of *Phyllanthus niruri*

Ethanol was used as blank and ethanolic DPPH was used as control. L-ascorbic acid-0.360; ethanolic DPPH (0.1 mM)-0.793. Percentage scavenging activities are shown in Table 5.

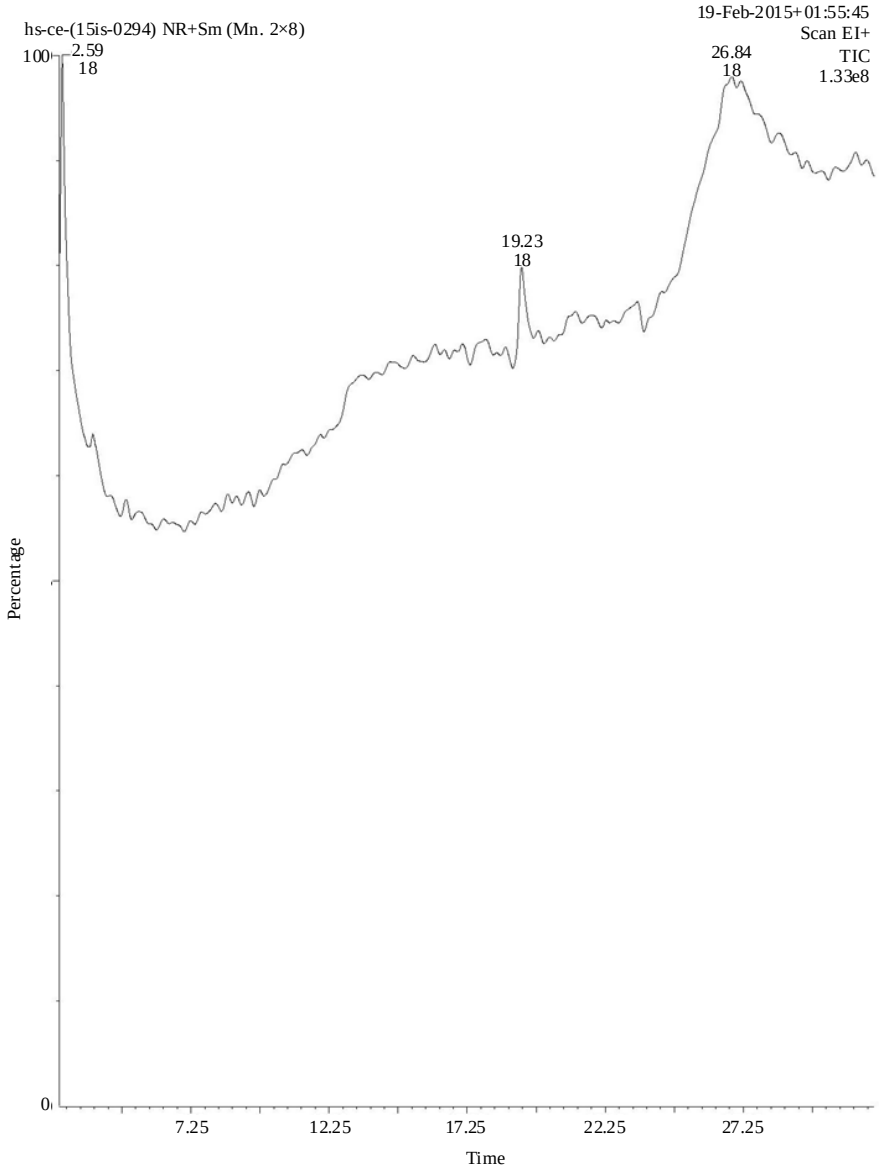

Fig. 5: Chromatogram of ethanol extract of *Cadaba fruticosa*

Table 4: Absorbance (O.D.) measured at 517 nm by DPPH method

Samples	D	E	F
*O. sanctum*_Eth	0.248	0.276	0.656
*O. sanctum*_Meth	0.144	0.251	0.683
*P. nirui*_Eth	0.239	0.277	0.553
*P. nirui*_Meth	0.164	0.256	0.532
*C. fruticosa*_Eth	0.202	0.232	0.732
*C. fruticosa*_Meth	0.207	0.228	0.669

In vitro anti-inflammatory activity

HRBC method: The HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of

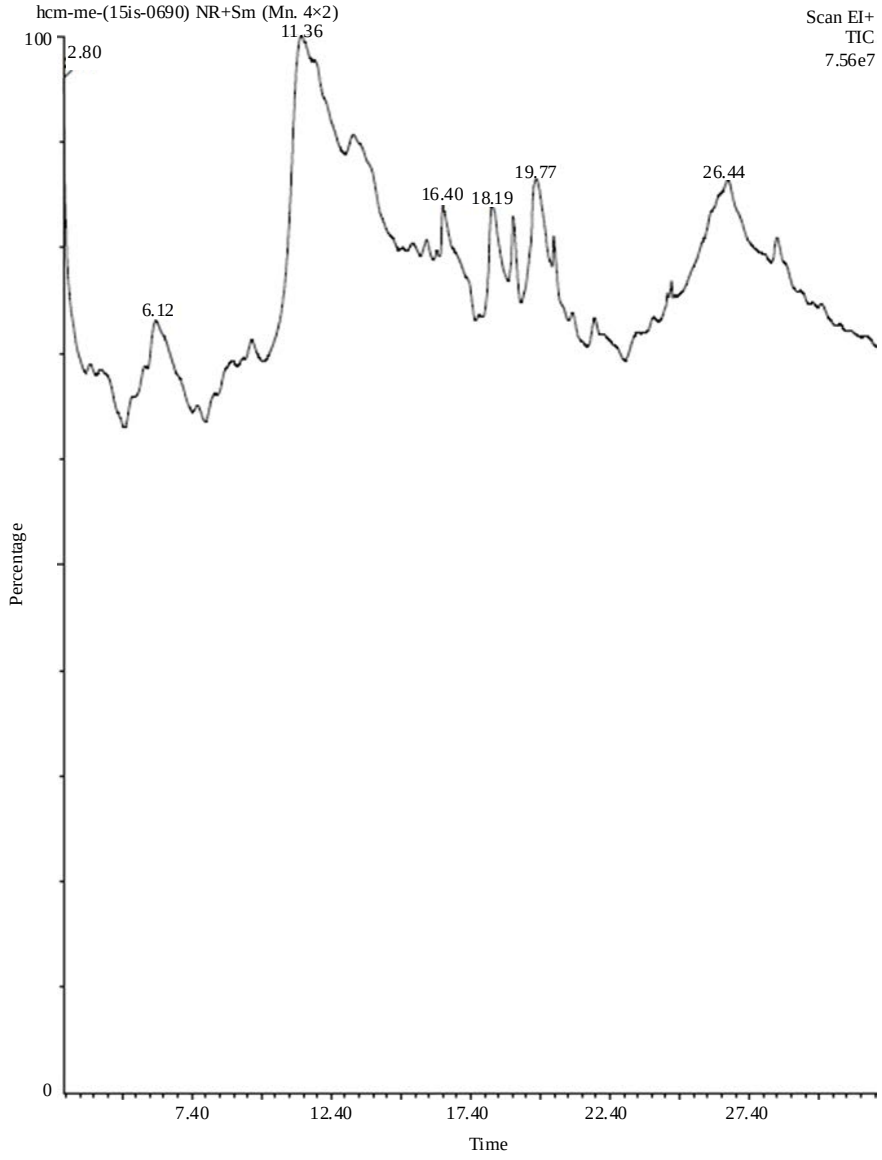

Fig. 6: Chromatogram of methanol extract of *Cadaba fruticosa*

Table 5: Percentage of scavenging activity of extracts, L-ascorbic acid-54.6%

Samples	D (%)	E (%)	F (%)
*O. sanctum*_Eth	68.7	65.19	17.2
*O. sanctum*_Meth	81.8	68.3	13.9
*P. nirui*_Eth	69.9	65.0	30.2
*P. nirui*_Meth	79.3	67.7	33.0
*C. fruticosa*_Eth	74.5	70.4	7.7
*C. fruticosa*_Meth	73.8	71.2	15.6

anti-inflammatory activity. Percentage cell stabilizing effects of the three herbs and the standard anti-inflammatory drug diclofenac are shown in Table 6-7. Percentage cell haemolysis of the three herbs is shown in Table 8.

Table 6: Percentage of cell stabilization using *Ocimum sanctum*, *Phyllanthus niruri* and *Cadaba fruticosa* extract

Concentration (µg mL^{-1})	Ethanol extracts (%)	Methanol extracts (%)	Aqueous extracts (%)
Ocimum sanctum			
25.5	63.99±0.64	55.17±0.56	12.38±0.12
50	67.30±0.73	61.10±1.11	13.15±0.76
100	69.96±0.81	63.10±0.97	13.70±1.02
200	82.70±0.92	76.07±1.21	21.38±0.63
Phyllanthus niruri			
25.5	63.50±0.64	60.18±0.56	11.81±0.12
50	65.64±0.73	60.77±1.11	41.18±0.76
100	80.71±0.81	71.40±0.97	29.80±1.02
200	86.71±0.92	81.64±1.21	80.57±0.63
Cadaba fruticosa			
25.5	66.91±0.64	59.84±0.56	13.86±0.12
50	70.84±0.73	61.21±1.11	15.42±0.76
100	81.80±0.81	66.38±0.97	16.01±1.02
200	84.04±0.92	66.39±1.21	31.53±0.63

Table 7: Comparison using standard drug

Concentration (µg mL^{-1})	Diclofenac
100	49.32±9.18
200	78.15±0.35

Table 8: Percentage of cell haemolysis using *Ocimum sanctum*, *Phyllanthus niruri* and *Cadaba fruticosa* extract

Concentration (µg mL^{-1})	Ethanol extracts (%)	Methanol extracts (%)	Aqueous extracts (%)
Ocimum sanctum			
25.5	36.00	44.82	87.52
50	30.01	38.12	86.85
100	17.29	38.10	86.29
200	12.29	36.90	78.62
Phyllanthus niruri			
25.5	36.50	39.82	88.19
50	34.36	38.90	87.60
100	19.28	28.60	70.20
200	13.60	18.36	58.82
Cadaba fruticosa			
25.5	33.08	40.16	86.14
50	29.15	38.74	86.14
100	18.19	38.37	84.57
200	15.96	33.60	68.46

GC-MS analysis: The analysis of the extracts of *Occimum sanctum, Phyllanthus niruri* and *Cadaba fruticosa* revealed the presence of several bioactive compounds and their respective beneficial effects in various disease conditions were identified using evidence from literature (Table 9-11).

DISCUSSION

Antioxidants play an important role in the disease prevention of the human body by virtue of scavenging free radicals. From cancer to immune system decline to ageing, antioxidants as vitamins, carotenoids, phenolic compounds, all have disease preventive capacity (Aksoy *et al*., 2013). By improving or enhancing the proliferation of T-Lymphocyte cells, antioxidants help in fighting off bacterial, viral, fungal infections. In this research, a comparative study of three medically important herbs has been conducted. Previously much research has been done on *Ocimum sanctum* and *Phyllanthus niruri*. The study on *Cadaba fruticosa*, a herb yet to be scrutinized, has yielded promising results in terms of antioxidant capacity, scavenging activity, antimicrobial and anti-inflammatory responses (Subbaiah and Savithramma, 2013; Mythreyi *et al*., 2009).

Table 9: List of some bioactive compounds isolated and detected using GC-MS in *Ocimum sanctum* and their potential applications

Plant	Bioactive compounds	Functionality
Ocimum sanctum	9-octadecynoic acid	Aids in reducing blood pressure (hypotensive effect)
	8,11,14-eicosatrienoic acid, (Z,Z,Z)-, Isopropyl linoleate	Has beneficial effects on the skin
	Squalene	A terpenoid used in the synthesis of steroids and is used as an immunological adjuvants in vaccines for humans Also a chemopreventive substance, used for protecting people from cancer
	Geranylgeraniol (Diterpene)	Potent inhibitor of *Mycobacterium tuberculosis* (*in vitro*)
	Farnesol	An acyclic sesquiterpene alcohol which has antibacterial effects against *Staphylococcus aureus*
	5-hydroxy-4-octanone	A flavoring agents in the food industry belongs to family of acyloins
	N-acetyl-3-methoxyamphetamine Methoxyamphetamine	Used as a stimulant drug for humans
	Estragole (Phenylpropene)	Chemically important essential oil reported to be genotoxic and carcinogenic
	D-alanine	An essential amino acid: used in peptide antibiotics
	N-methylglycine (sarcosine)	Important amino acid used in the treatment of schizophrenia and used as a prostate cancer marker
	N'-isopropylureidoacetic acid	Commonly used as a herbicide

Table 10: List of some bioactive compounds detected in *Phyllanthus niruri* and their potential applications

Plant	Bioactive compounds	Functionality
Phyllanthus niruri	Gamolenic acid (GLA)	Commonly used in vegetable oils and is sold as dietary supplements for various health problems Also used in the treatment of breast pain and eczema
	4-methyl-2,5-dimethoxyphenethylamine	A psychedelic drug which yet needs to be investigated
	Phenol, 3-methoxy-2,4,6-trimethyl-; Phenol, 5-methoxy-2,3,4-trimethyl	Phenolic bioactive compound indicating its antioxidant activities
	Phenethylamine, 2-methoxy-.alpha.-methyl-4,5 (Methylenedioxy)-derivative of phenethylamines	Used in psychoactive drugs that act as entactogens, psychedelics and/or stimulants, as well as entheogens
	Sylvatesmin	A ligand found in herbal plants and accounts for high scavenging activity
	2H-pyran-2-one, 6-[2-(3,4 dimethoxyphenyl) Ethyl-5,6-dihydro-4-metho: Derivative of Pyranone	Maybe used to treat retrovirus infection
	Dihydrocapsaicin, N-methyl-, methyl ether	An irritant usually found in chili peppers

Table 11: List of some bioactive compounds detected in *Cadaba fruticosa* and their potential applications

Plant	Bioactive compounds	Functionality
Cadaba fruticosa	Hydroxyurea	An antineoplastic drug Reduce the rate of painful attacks in sickle-cell disease Has antiretroviral properties in diseases such as HIV/AIDS
	1-amino-2-propanol	An intermediate in the synthesis of a variety of pharmaceutical drugs It's a basic building block of the opioid methadone
	2-hexanamine, 4-methyl (derivative of methylhexanamine)	Used as an indirect sympathomimetic drug that constricts blood vessels and thus has effects on the heart, lungs, and reproductive organs Causes bronchodilation, inhibits peristalsis in the intestines and has diuretic effects
	Derivatives of dodecenol	Used as food additives, cosmetics and emollient in pharmaceutical industries
	Derivatives of imidazoles	Used as antifungal drugs
	Isoglutamine	Derivative of muramyl dipeptide can used to treat osteosarcoma

The antimicrobial tests indicated that alcoholic extract of *Ocimum sanctum* showed maximum growth inhibition against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Alcoholic extract of *Phyllanthus niruri* showed maximum growth inhibitory effect against *Escherichia coli*. Aqueous extracts of both were almost minimal. Whereas, *Cadaba fruticosa*, in all three extract forms showed the high potency against all three strains.

The antioxidant activity tests revealed that along with *Ocimum sanctum* and *Phyllanthus niruri*, *Cadaba fruticosa* showed very good results in terms of antioxidant capacity and scavenging activity. This was comparable with previous studies (Lavinya *et al*., 2014). The reducing power of extracts is due to presence of bioactive compounds which essentially possess donating abilities which proves its strong reducing power potential (Wong *et al*., 2006). In the DPPH assay, antioxidants present naturally in the plant extracts were able to reduce stable free radical of DPPH the pale yellow colour of 1, 1-diphenyl-1, 2-picryl hydrazine when it reacts with hydrogen donors. Methanolic extract of *Ocimum sanctum* at 200 μg mL^{-1} depicted highest scavenging activity, 81.1%. The methanol extracts showed a slightly higher activity than ethanol extracts. The assays confirmed the strong antioxidant properties of the three herbal plants. Hence proving that *C. fruticosa* can be pharmacologically and clinically active (Arokiyaraj *et al*., 2008).

During inflammation, lysosomal hydrolytic enzymes are released into the sites which cause damage of the surrounding organelles and tissues, creating variety of disorders. Protective effect on heat and hypotonic saline induced erythrocyte lysis is known to be a very good index of anti-inflammatory activity for any sample. The *in vitro* anti-inflammatory tests by Human Red Blood Cells (HRBC) membrane stabilization method was selected because of its simplicity and reproducibility. Among the three plants with four different concentrations in three solvents, ethanol extract of *Phyllanthus niruri*, at 200 μg mL^{-1}, showed 86.7% protection of HRBC in hypotonic solution. This was closely followed by ethanol extract of *Cadaba fruticosa* (84%) and then ethanol extract of *Ocimum sanctum* at 82%. The results were compared by standard dicolfenac sodium which has a standard value of 78.2% at 200 μg mL^{-1}. Such activity may be the presence of certain phytochemical constituents which helped in the inhibition of hypotonic induced cell lysis. This was also proved that with increase concentration, cell haemolysis percentage also decreased, hence the anti-inflammatory activity of all the plants were concentration dependent (Anosike *et al*., 2012).

The GC-MS analysis showed the presence of various bioactive compounds in the three herbal plants. *Ocimum sanctum*, contained pharmacologically important biologically active compounds like octadenoic acids, squalene, farnesol, geranylgeraniol and many more. Derivative of methoxyamphetamine was detected which can be used as a stimulant drug for humans; sarcosine was also present which is a therapeutically potent drug used to treat schizophrenia (Lane *et al*., 2010).

Phyllanthus niruri, interestingly traces of psychoactive elements were detected. 4-methyl-2,5-dimethoxyphenethylamine, phenethylamine, 2-methoxy-.alpha.-methyl-4,5 (methylenedioxy)-derivative of phenethylamines have been reported to have psychedelic effect on humans. Further investigation is needed to prove the effectiveness of *Phyllanthus* species as a potent psychoactive drug source.

Cadaba fruticosa, showed high amounts of hydroxyurea, used as an antineoplastic drug, reduces rate of painful attacks in sickle-cell disease and has antiretroviral properties in diseases such as HIV/AIDS. Various derivatives of imidazoles were also detected thereby indicating antifungal activity of *C. fruticosa*. This analysis showed the existence of various compounds with different chemical structures. The occurrence of various bioactive compounds proves the purpose of *Ocimum sanctum, Phyllanthus niruri* and *Cadaba fruticosa* for various disorders. However, seclusion of individual phytochemical constituents may proceed to find an innovative drug.

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