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COMPARATIVE PHYTOCHEMICAL AND BIOLOGICAL EVALUATION OF ANTIMICROBIAL, ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF EXTRACTS FROM THE LEAVES OF *LANTANA CAMARA* AND *PIPER NIGRUM*

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ABSTRACT

Comparative analysis and confirmation of Phytochemicals from leaves of *Lantana camara* (*L.camara*) and *Piper nigrum* (*P.nigrum*). Evaluation of their antimicrobial, antioxidant and antidiabetic activity. Plant extracts obtained from *Lantana camara* and *Piper nigrum* using different solvents- hexane, benzene, chloroform, methanol and distilled water by soxhlet apparatus were subjected to preliminary phytochemical analysis and confirmation by Gas Chromatography and Mass Spectroscopy (GC-MS). Methanol extract further subjected to determine antimicrobial activity using Disc diffusion method, antioxidant activity by Ferric ion reducing antioxidant power assay (FRAP) and 1,1 diphenyl 2-Picryl Hydrazil free radical scavenging assay (DPPH) and antidiabetic activity by α-amylase inhibition assay. For all the above samples were assayed in triplicates. Phytochemical screening and GC-MS analysis confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, phenols, carbohydrates, amino acids and proteins, glycosides, saponins and phytosterols in different extracts of *Lantana camara* and *Piper nigrum*. The methanol extract of *L. camara*, showed inhibition zone against the pathogens – *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Aspergillus niger*. The methanolic extract of *P. nigrum* showed zone of inhibition against the pathogen *Corynebacterium.* FRAP method revealed the antioxidant activity of methanol extract of 2 plant at 50 µg/ml in increasing order *Lantana camara:* 3.217 ± 0.010 and *Piper nigrum:* 2.472 ± 0.015 at 700nm using 50 µg/ml of the Standard ascorbic acid 3.687±0.004. The anti-oxidant activity of plant extracts were less compared to standard. DPPH results revealed that the % of scavenging activity at 750nm using 50 µg/ml was 75.21± 0.486 of *Lantana camara* and low when compared to standard 89.32 ± 0.476 but high then that of *Piper nigrum*. Anti-diabetic assay of methanolic extract of *Lantana camara* and *Piper nigrum*, *α*- amylase enzyme inhibitory activity was IC_{50} 13.68 ± 0.05 and 28.67 ± 0.23µg /ml respectively with reference to standard acarabose IC_{50} of 0.32 µg /ml under similar experimental conditions. The present study indicates that *Lantana camara* and *Piper nigrum* is highly abundant in secondary metabolites and of Pharmacological importance showing antimicrobial, antioxidant and antidiabetic activity.

KEYWORDS: *Lantana camara*, *Piper nigrum*, Anti-diabetic assay, Phytochemical screening.

INTRODUCTION

Plants are rich in wide range of secondary metabolites useful therapeutically (Srivastava C. 2009). Research proves the multidrug resistance by Pathogens- microbes and increasing need for new antimicrobial drug (Hemaiswarya *et al.,* 2009). Plants are a rich source with potential for discovery of new drug- antimicrobial, antioxidant and antidiabetic activity (Aswar *et al.,* 2008).

Lantana camara is also used as folk remedies to treat cancer tumours, chicken pox and measles. The roots being rich with olenolic acid and leaves contain umuhengerin responsible for antimicrobial activity (Badakhshan *et al.,* 2012).

Piper nigrum contain piperine first pharmacologically active compound isolated from Piperaceae family also indicated presence of phenolic, flavonoids, alkaloids, amides, terpenes, steroids etc and proved to be good source for antimicrobial and antioxidant activity (Silva, *et al.,* 2008) (Valle *et al.,* 2016).

Research proves plants as life saver in coming days (Sinem *et al.,* 2015) Phytochemical analysis is intended to serve as abundant source for information on analytical and instrumental methodology in the plant sciences (Saad *et al.,* 2011). Rich in phytochemicals that can be implemented for anti-inflammatory (Sreelekshmi *et al.,* 2007) antidiabetic, anti-cancerous activity (Pandit *et al.,* 2016) (Jung *et al.,* 2014).

2. MATERIALS AND METHODS

2.1. Collection of sample

The plant of *Lantana camara* (family: Verbeneceae) was collected from Alnavar, district Dharwad, Karnataka; *Piper nigrum* (Family: Piperaceae), was collected from Kumta, Karnataka; India in September 2017. The plants were identified and authenticated by Dr. Kotresha K. (Taxonomist) *International Journal of Medicinal Plants*, Department of Botany-Karnatak Science College, Dharwad.

2.2. Pre-treatment and Extraction

The plant leaves were washed under running tap water, surface sterilized using Sodium hypochlorite (0.9%) and washed with double distilled water. The leaves were cut into small pieces using sterile scalpel, shade dried at room temperature and powdered mechanically. 100 g of the powdered leaves was subjected to solvent extraction with 250 ml of Hexane boiling Point (BP: 69˚C), Benzene (BP: 80.1˚C), Chloroform (BP: 61.2˚C), Methanol (BP: 64.6˚C) and water (BP: 100˚C) by solvent extraction method using soxhlet apparatus. The extract collected was filtered and evaporated using rotary flask evaporater and stored in air tight bottle at 4˚C for further use (Surendra K *et al.*, 2012).

2.3. Preliminary phytochemical screening

The crude leaf extract of plant sample were subjected to qualitative biochemical tests for identification of preliminary phytochemical constituents such as alkaloid, amino acids, carbohydrates, flavonoids, phytosterols, phenolics, reducing sugar, saponins, tannins and terpenoids according to standard procedures (Ayoola GA *et al.*, 2008).

2.4. Chemical composition analysis

Methanol extract obtained from leaves of *Lantana camara* and *Piper nigrum* was used for the determination of bioactive compounds by Gas Chromatography-Mass Spectroscopy (GC-MS) in University Science Instrumentation Centre (USIC) at Karnatak University Dharwad. Analysis was done using GCMSQP2010S instrument.

2.5. Tested microorganism

Gram positive bacterial strains of *Staphylococcus aureus* (*S. aureus*), *Corynebacterium* and *Bacillus subtilis* (*B. subtilis*); Gram negative bacterial strains of *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Aerobacter aerogenes* (A. aerogenes), and fungal strain of *Candida albicans* (*C. albicans*) and *Aspergillus niger* (*A. niger*) were used as test microorganisms to determine the antibacterial and antifungal activity of plant methanol extract by disc diffusion method (Mohanta T.K *et al.*, 2007). The parent cultures of microorganisms were maintained in the Department of Biotechnology and Microbiology, Karnatak University, Dharwad (collected from Hubli diagnostics).

2.6 Disc diffusion method

The obtained methanolic plant extract was dissolved in 1% DMSO at the concentration of 10 mg/ml for preparation of stock. To evaluate the antimicrobial activity, disc diffusion method was used. The bacterial and fungal suspension culture was made by diluting in autoclaved distilled water (5 ml) for each test microorganism, 1 ml of each bacterial suspension culture and fungal suspension culture was poured and spread on to the different Mueller-Hinton agar plates and Czapadex agar plates respectively, for uniform distribution of microorganisms.

The methanol crude leaf extracts from the stock was poured on sterile Whatmanns filter paper No.40, disc- 3 mm in diameter, using a sterile micropipette and dried to obtain varying concentration i.e., 0.05, 0.1, 0.15 and 0.2 mg/disc. Streptomycin (0.1 mg/disc) was used as standards positive control agents for bacteria. Nystatin (0.1 mg/disc) was used as a positive control agent for fungus followed by 1% DMSO as negative control. The Mueller-Hinton agar plates was incubated for 18 hrs at 37ºC for bacterial growth and Czapadex agar plates incubated for 36 hrs at room temperature for fungal growth. At the end of the incubation period, the zone of inhibition was measured (Arun Kumar and Muthuselvam 2009).

2.7 Ferric ion reducing antioxidant power assay (FRAP)

Ferric ion reducing power was measured according to the method of (Benzie *et al*., 1996). Methanol extract of plant leaf sample was taken in the concentration ranging from 10 µg/ml to 50 µg/ml and was mixed with 2.0 ml of 20 mM phosphate buffer and 2.0 ml of 1%, w/v potassium ferricyanide. The reaction mixture was incubated at 50ºC for 20 min. After incubation 1.0 ml of 10%, w/v trichloroacetic acid (TCA) and 0.4 ml of 0.1%, w/v ferric chloride was added to the mixture. The reaction mixture was incubated for 10 minute. The absorbance was measured at 700 nm using UV spectrophotometer. Ascorbic acid was used as a standard reference. All the samples were assayed in triplicates.

2.8. DPPH (1, 1 diphenyl 2 – Picryl Hydrazil) free radical scavenging assay

Free radical scavenging activities of methanol extract of *plant* leaf were determined using DPPH radical as a reagent, according to the method of (Rice-Evans *et al.*, 1997). Ascorbic acid was used as a standard reference. 100 µl of DPPH radical solution in methanol (60 µM) was mixed with 100 µl of sample extract in methanol (different concentration, w/v). The mixture was incubated at room temperature in dark condition for about 30 min and the absorbance measured at 517 nm using UV-VIS.

Spectrophotometer. All samples were assayed in triplicates.

The DPPH scavenging activity of each sample was calculated using the formula:
% Inhibition = A_t-A_c X 100/ A_c Where,
A_c is the absorbance of the control reaction i.e. 100 µl of methanol with 100 µl of the DPPH solution.
A_t is the absorbance of the test sample.

The IC_{50} value was calculated for plant extract. Higher free radical activity is directly proportional to lower absorbance of the reaction mixture.

2.9. α- amylase inhibition assay (Anti-diabetic activity)
α -amylase activity was carried out by starch-iodine method. 10 µl of α-amylase solution (0.025 mg/ml) was mixed with 390 µl of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of methanol plant extracts. After incubation at 37°C for 10 min, 100 µl of starch solution (1%) was added, and the mixture was re-incubated for 1 hour. After incubation 0.1 ml of 1% iodine solution and 5 ml distilled water was added to the reaction mixture, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as,
Percentage % = (A-C) X 100 / (B-C)

Where, A= absorbance of the sample, B= absorbance of blank (without α-amylase), and C = absorbance of control without starch.

3. RESULTS

3.1. Preliminary phytochemical determination
The preliminary phytochemical qualitative analysis revealed the presence of secondary metabolites flavonoids, phenols, carbohydrates, amino acids and proteins, phytosterols in hexane extract; Phenols and carbohydrates in benzene extracts; alkaloids, flavonoids, tannins, phenols, carbohydrates, amino acids and proteins in methanol extract; flavonoids, terpenoids, phenols and carbohydrates in chloroform extract; alkaloids, flavonoids, terpenoids, carbohydrates, amino acids, proteins, glycosides and saponins, in aqueous extract of *Lantana camara*.

The presence of secondary metabolites terpenoids, phenols, glycoside and phytosterols in hexane extract; terpenoids, phenols and glycosides in benzene extract; alkaloids, flavonoids, terpenoids, carbohydrates, amino acids, proteins, glycosides and phytosterols in methanol extract; flavonoids, tannins, terpenoids, phenols, carbohydrates and glycosides in chloroform extract; flavonoids; terpenoids, phenols, carbohydrates, amino acids, proteins, glycosides and saponins in aqueous extract of *Piper nigrum*.

3.2. Chemical composition analysis by Gas chromatography- mass spectroscopy (GC-MS)
The GC-MS analysis was carried out for methanol extract of plant sample. The list of molecules was identified by referring "National Institute of Standard and Technology" (NIST) library based on the name of compound, common name, molecular formula, molecular weight, retention time, peak area, compound nature.

3.3. Antimicrobial activity of solvent extract
Gram positive bacterial strains of *Staphylococcus aureus, Corynebacterium and Bacillus subtilis*, Gram negative bacterial strains of *Escherichia coli*, *Klebsiella pneumoniae and Aerobacter aerogenes*, and fungus *Candida albicans and Aspergillus niger*, were used as test microorganism to determine the antibacterial and antifungal activity of 2 different plant extracts by disc diffusion method (Arun Kumar and Muthuselvam 2009).

The methanolic extracts of *Lantana camara* at 0.05, 0.1, 0.15, and 0.20 mg/disc, showed zone of inhibition (1.0,1.0,5.0 and 5.0 mm) against *Staphylococcus aureus*, (2.0, 3.0, 5.0, and 2.0mm) against *Bacillus subtilis*, (1.0, 1.0.1.0 and 2.0) against *E.coli* (1.0, 3.0, 4.0 and 4.0) against *Aspergillus niger* followed by *Piper nigrum* (0.0, 1.0, 2.0 and 4.0 mm) against *Corynebacterium*. Streptomycin (0.1 mg/disc) was used as standards positive control agents for bacteria. Nystatin (0.1 mg/disc) was used as a positive control agent for fungus followed by 1% DMSO as negative control.

3.4. Ferric ion reducing antioxidant power assay (FRAP)
By FRAP method the antioxidant activity of the Standard ascorbic acid exhibited higher absorbance at 50 µg/ml i.e 3.687±0.004 at 700 nm, with reference to standard the antioxidant activity of methanolic extract of 2 plant at 50 µg/ml in increasing order is *Lantana camara:* 3.217 ± 0.010, *Piper nigrum:*2.472 ± 0.015. The anti-oxidant activity of plant extracts were less compared to standard.

3.5. DPPH (1, 1 diphenyl 2 – Picryl Hydrazil) free radical scavenging assay
The antioxidant activity of methanolic extracts of plants was compared with Ascorbic acid as standard antioxidant. The results revealed that the % of scavenging activity 75.21± 0.486 of *Lantana camara* was low when compared to standard ascorbic acid 89.32 ± 0.476 but high then that of *Piper nigrum*. The scavenging activity of methanol extract increased with increase in concentration.

3.6. α- amylase inhibition assay (Anti-diabetic activity)
Anti-diabetic assay of methanolic extract of *Lantana camara* and *Piper nigrum, α*- amylase enzyme inhibitory activity was IC_{50} 13.68 ± 0.05 and 28.67 ± 0.23µg /ml respectively with reference to standard acarabose IC_{50} of 0.32 µg /ml under similar experimental conditions.

4. Tables

4.1 Phytochemicals present in various solvents of *Lantana camara.*

Sl. No.	Name of Compound	Hexane	Benzene	Methanol	Chloroform	Water
1	Alkaloids	-	-	+	-	+
2	Flavonoids	+	-	+	+	+
3	Tannins	-	-	+	-	-
4	Terpenoids	-	-	-	+	+
5	Phenols	+	+	+	+	-
6	Carbohydrates	+	+	+	+	+
7	Amino acids and proteins	+	-	+	-	+
8	Glycosides	-	-	-	-	+
9	Saponins	-	-	-	-	+
10	Phytosterols	+	-	-	-	-

4.2 Phytochemicals present in various solvents of *Piper nigrum.*

Sl. No.	Name of Compound	Hexane	Benzene	Methanol	Chloroform	Water
1	Alkaloids	-	-	+	-	-
2	Flavonoids	-	-	+	+	+
3	Tannins	-	-	-	+	-
4	Terpenoids	+	+	+	+	+
5	Phenols	+	+	+	+	+
6	Carbohydrates	-	-	+	+	+
7	Amino acids and proteins	-	-	+	-	+
8	Glycosides	+	+	+	+	+
9	Saponins	-	-	-	-	+
10	Phytosterols	+	-	+	-	-

+ (present); - (absent) All samples analysed in triplicates.

Table 4.3. Compounds obtained by GC-MS profiling (as referred to NIST library) in methanol extract of *Lantana camara.*

Sl. No	Compound name	Molecular Formula	Molecular weight	Retention time	Peak area	Compound nature	Uses
1.	Benzaldehyde, 3,hydroxy-9-methoxy	C8H8O2	136.1	11.9	0.232	-	-
2.	Bicyclo(3,1,1) heptane-2,3-diol 2,6,6 trimethyl (2,3 184.36Pinanediol	C10H18O2	170.0	13.5	0.97	Terpene	Antimicrobial
3.	Tridecane	C13H28	184.36	14.7	-	Hydrocarbon	Antidiabetic
4.	1-dodecanol	C12H26O	186.0	15.2	4.83	Long chain fattyacid	Antibacterial and Antidiabetic

Table 4.4. Compounds obtained by GC-MS profiling (as referred to NIST library) in methanol extract o*f Piper nigrum.*

Sl. No	Compound Name	Molecular Formula	Molecular Weight	Retention Time	Peak Area	Compound Nature	Uses
1.	Bicyclo(3,1,1) heptane2,3-diol 2,6,6, trimethyl (2,3 Pinanediol	C10H18O2	170.0	13.5	0.97	Terpene	Antimicrobial
2.	-	-	-	15.1	-	-	-
3.	-	-	-	15.3	-	-	-
4.	Cyclotetradecane	C14H28	196.37	17.7	-	-	Antidiabetic
5.	Pentadecane	C15H32	212.42	18.0	-	-	-
6.	2,-Propanol, 1,1-(1methyl-1,2-ethanedidyl)bis (oxy bis(Tripropylene glycol)		192.0	18.1	0.87	-	Antimicrobial
7.	1,3 Cyclohexadione, 2 methyl-5-(1-methylethyl)-(1-phellandrene)		186	22.5	0.23	Monoterpene	Antibacterial

Table 4.5. Antimicrobial activity of methanolic extract of *Lantana camara* and *Piper nigrum*.

Methanolic extract (0.05, 0.1, 0.15 and 0.2 mg/disc) exhibiting zone of inhibition in mm										
Microbial species	**Streptomycin**	**Nystatin**	***Lantana camara***				***Piper nigrum***			
Gram positive bacteria										
Staphylococcus aureus	7.0	-	1.0	1.0	5.0	5.0	-	-	-	-
Corynebacterium	5.0	-	-	-	-	-	0.0	1.0	2.0	4.0
Bacillus subtilis	7.0	-	2.0	3.0	5.0	2.0	-	-	-	-
Gram negative bacteria										
Escherichia coli	4.0	-	1.0	1.0	1.0	2.0	-	-	-	-
Klebsiella pneumoniae	2.0	-	-	-	-	-	-	-	-	-
Aerobacter aerogenes	4.0	-	-	-	-	-	-	-	-	-
Fungi										
Candida albicans	-	8.0	-	-	-	-	-	-	-	-
Aspergillus niger	-	12.0	1.0	3.0	4.0	4.0	-	-	-	-

Table 4.6. Ferric ion reducing antioxidant power assay (FRAP): Methanol extract of plant sample at optical density 700 nm.

Sl. No	Volume in µl	Standard Ascorbic acid	*Lantana camara*	*Piper nigrum*
1.	10	3.164 ± 0.003	2.614 ± 0.006	2.113 ± 0.005
2.	20	3.259 ± 0.001	2.926 ± 0.005	2.215 ± 0.004
3.	30	3.418 ± 0.003	3.161 ± 0.003	2.311 ± 0.005
4.	40	3.546 ± 0.002	3.203 ± 0.015	2.398 ± 0.006
5.	50	3.687± 0.004	3.217 ± 0.010	2.472 ± 0.015

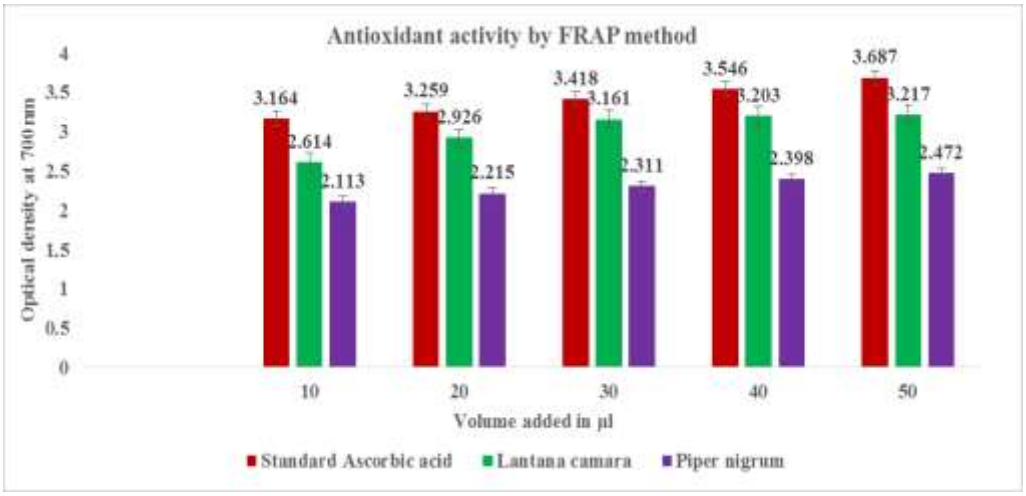
Table 4.7 DPPH (1,1 diphenyl 2 –Picryl Hydrazil) Free radical scavenging assay: Methanol extract of plant sample at optical density 715 nm.

Sl. No	Concentration In µg	Standard Ascorbic acid	*Lantana camara*	*Piper nigrum*
1.	10	71.21 ± 0.121	53.14 ± 0.110	49.78 ± 0.225
2.	20	77.64 ± 0.198	57.58 ± 0.015	51.83 ± 0.265
3.	30	82.54 ± 0.187	63.29 ± 0.223	51.91 ± 0.200
4.	40	84.85 ± 0.011	68.99 ± 0.145	54.76 ± 0.015
5.	50	89.32 ± 0.476	75.21± 0.486	57.90 ± 0.469

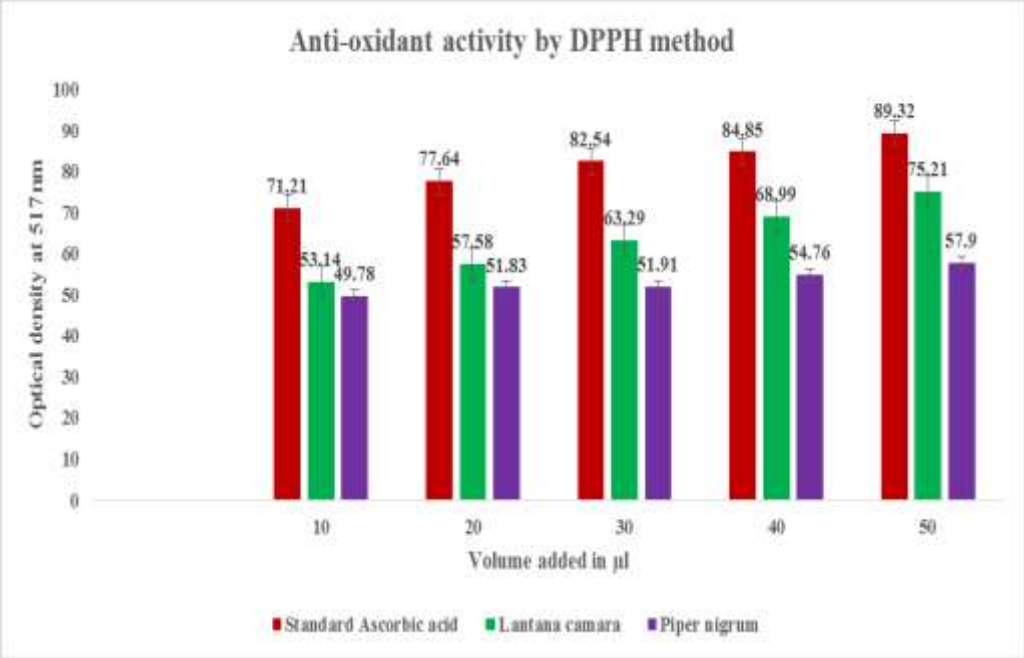
Table 4.8: Comparison of the IC_{50} in µg /ml of extract against α- amylase enzyme inhibition. Extract IC_{50} in µg/ml

Plant Extract	IC_{50} in µg /ml
Lantana camara	13.68 ± 0.05
Piper nigrum	28.67 ± 0.23
Acarabose	0.32 ± 3.2

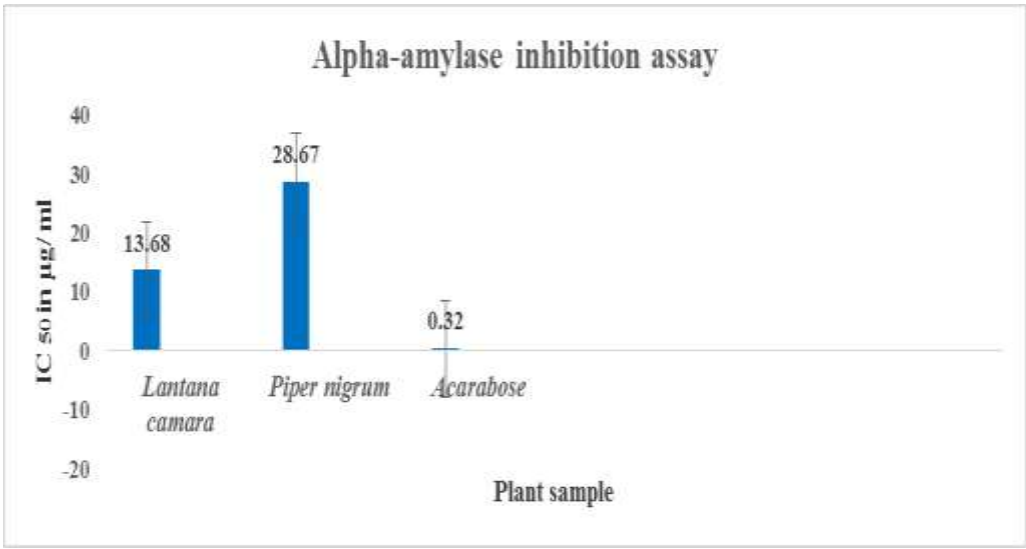
5. Graphs



Graph 1: Ferric ion reducing antioxidant power assay (FRAP).



Graph 2: (1,1diphenyl 2- picryl Hydrazil) free radical scavenging assay.



Graph 3: Comparison of the IC_{50} in µg /ml of extract against α- amylase enzyme inhibition. Extract IC_{50} in µg/ml.

DISCUSSION

Plants are Potential renewable natural resource of phytochemicals which is of medicinal value and beneficial for human health care (Srivastava C. 2009). The present study indicates presence of alkaloids, flavonoids, tannins, terpenoids, phenols, carbohydrates, amino acids and proteins, glycosides, saponins and phytosterols in different extracts of *Lantana camara* and *Piper nigrum*.

The further confirmation by GC-MS reveals pharmacological activity. Methanol extract of *Lantana camara* consist of Bicyclo(3,1,1)heptane-2,3-diol2,6,6trimethyl(2,3 184.36Pinanediol) responsible for antimicrobial activity hence shows zone of inhibition against pathogens *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Aspergillus niger*, the presence of Tridecane and 1-dodecanol indicates presence of antioxidant and antidiabetic activity. The GC-MS analysis of Methanol extract of *Piper nigrum* confirms presence of Bicyclo(3,1,1) heptane2,3-diol2,6,6,trimethyl(2,3 Pinanediol), Cyclotetradecane and 1,3 Cyclohexadione,2 methyl-5-(1-methylethyl)-(1-phellandrene) confirms antimicrobial activity and justifies the antibacterial activity against *Corynebacterium*.

The role of free radical reactions in disease pathology is well studied and is known to be involved in many acute and chonic disorders, such as diabetes, atherosclerosis, aging, immune suppression etc. Due to the presence of the conjugated ring structures and hydroxyl groups many

phenolic compounds have the potential to function as antioxidants by scavenging or stabilizing free radicals involved in the oxidation process through hydrogenation or complexing with oxidizing species (Afsar *et al.*,2018*)*. Plants comprising phenolic constituents such as phenolic compounds. Flavonoids, phenolic acids, tannins are possible souce of natural antioxidants (Ndam *et al.*,2016*)*. By FRAP method revealed the antioxidant activity of methanol extract of 2 plant at 50 µg/ml in increasing order *Lantana camara:* 3.217 ± 0.010 and *Piper nigrum:* 2.472 ± 0.015 at 700nm using 50 µg/ml of the Standard ascorbic acid 3.687±0.004. The anti-oxidant activity of plant extracts were less compared to standard. DPPH results revealed that the % of scavenging activity at 750nm using 50 µg/ml was 75.21± 0.486 of *Lantana camara* and low when compared to standard 89.32 ± 0.476 but high then that of *Piper nigrum*.

Diabetes characterized by chronic hyperglycaemia with disturbance of carbohydrates, fat and protein metabolism, resulting from defects in insulin secretion and uptake by cell. A number of active compounds are isolated from medicinal plants for direct use as a drug or act as a lead compound or pharmacological agent (Pandit *et al.*,2016*)*. In the present study the GC-MS analysis confirms presence of Tridecane and 1-dodecanol in methanolic extract of *Lantana camara* and Cyclotetradecane in *Piper nigrum* hence potential antidiabetic activity.

Anti-diabetic assay of methanolic extract of *Lantana camara* and *Piper nigrum, α*- amylase enzyme inhibitory activity was IC_{50} 13.68 ± 0.05 and 28.67 ± 0.23µg /ml respectively with reference to standard acarabose IC_{50} of 0.32 µg /ml under similar experimental conditions. The study conclude that the *Lantana camara* and *piper nigrum* is active source for secondary metabolites and pharmacological importance.

REFERENCES

1. Afsar, T., Razak, S., Shabbir, M., and Khan, M. R. (2018). Antioxidant activity of polyphenolic coumpounds isolated from ethyl-acetate fraction of *Acacia hydaspica* R. Parker. *Chemistry Central Journal*, 12(1): 5.
2. Arunkumar, S., & Muthuselvam, M. (2009). Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World Journal of Agricultural Sciences*, *5*(5): 572-576.
3. Aswar, M., Aswar, U., Wagh, A., Watkar, B., Vyas, M., & Gujar, K. N. (2008). Antimicrobial activity of *Ficusbenghalensis*. *Pharmacologyonline*, *2*: 715-725.
4. Ayoola, G. A., Coker, H. A., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., & Atangbayila, T. O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, *7*(3): 1019-1024.
5. Badakhshan M.P, Subramanion L Jothy, Sreenivasan Sasidharan (2012), Antioxidant activity of methanol extract of different parts of *Lantana camara*, *Asian Pacific Journal of Tropical biomedicine*, 960-965, DOI: 10.1016/S2221-1691(13)60007.
6. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1): 70-76.

i. Hemaiswarya, S., Poonkothai, M., Raja, R., & Anbazhagan, C. (2009). Comparative study on the antimicrobial activities of three Indian medicinal plants. *Egyptian Journal of Biology*, *11*(1).

7. Jung, I. L. (2014). Soluble extract from *Moringa oleifera* leaves with a new anticancer activity. *PloS one*, 9(4): e95492.
8. Kumar, R. S., Venkateshwar, C., Samuel, G., & Rao, S. G. (2013). Phytochemical screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. *International Journal of Engineering Science Invention*, *2*(8): 65-70.
9. Mohanta, T. K., Patra, J. K., Rath, S. K., Pal, D. K., & Thatoi, H. N. (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* Lf. *Scientific Research and Essays*, *2*(11): 486-490.
10. Nadam, L. M., Mih, A. M., Tening, A. S./ Fongod, A.G.N., Temenu, N. A., and Fujii, (2016). Phytochmemical analysis, antimicrobial and antioxidant activities of Euphorbia golondrina LC Wheeler (*Euphorbiaceae Juss*): an unexplored medicinal herb reported from Cameroon. *SpringerPlus*, 5(1): 264.
11. Pandit, R., Phadke, A., and Jagtap, A. (2010). Antidiabetic effect of *Ficus religiosa* extract in streptozotocin-induced diabetic rats. *Journal of ethno pharmacology*, 128(2): 462-466.
12. Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, *2*(4): 152-159.
13. Saad, S., Taher, M., Susanti, D., Qaralleh, H., and Rahim, N. A. B. A. (20110. Antimicrobial activity of mangrove plant (*Lumnitzera littorea*). *Asian Pacific journal of tropical medicine*, 4(7): 523-525.
14. Sinem Aslan Erdem, Seyed Fazel Nabavi, likay Erdogan Orhan, Maria Daglia, Morteza Izadi and Sayed Mohammad Nabavi, (2015), A review of phytochemical composition andantimicrobial activity of plants belonging to the genus *Erynfgium*, DARU *Journal of Pharmaceutical Sciences*, DOI: 10.1186/ s40199-015-016-3.
15. Sreelekshmi, R., Latha, P.G., Arafat, M.M., Shyamat, S., Shine, V.J., and Rajasekharan, S. (2007). Anti-inflammatory, analgesic and anti-lipid

peroxidation studies on stem bark of *Ficus religiosa Linn*.

16. Srivastava, C. (2009). Phytochemistry and medicobotany of some medicinal plants used in treatment of arthritis. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, *1*(1): 27-32.
17. Surendra K Rathore, Shashank Bhatt, Dhyani, Aanchal Jain (2012), Preliminary phytochemical screening of medicinal plant *Ziziphus mauritiana* Lam. Fruits, *International Journal of Current Pharmaceutical Research*, 4(3): 160-162, ISSN - 0975-7066.
18. Shakeel Ahmed Adhoni, Shivasharana Chandrabanda Thimmappa, Basappa Basawanneppa Kaliwal *, Phytochemical analysis and antimicrobial activity of *Chorella vulgaris* isolated from Unkal. *Journal of Coastal Life Medicine*, 2016; 4(5): 368-373.
19. Valle Jr, D. L., Puzon, J. J. M., Cabrera, E. C., and Rivera, W. L. (2016). Research Article Thin Layer Chromatography-Bioautography and Gas Chromatography- Mass Spectrometry of Antimicrobial Leaf Extracts from Philippine *Piper betle* L. against Multidrug-Resistant Bacteria.