International Journal of Scientific Research in Science, Engineering and Technology (www.ijsrset.com)
© 2020 IJSRSET | Volume 7 | Issue 1 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
DOI : https://doi.org/10.32628/IJSRSET20718

Comparative Analysis of a few Indian Medicinal Plants for their Antimicrobial and Antioxidant Properties

Kiran Kumar D. J., Bhanupriya H. J.
Department of P.G. Studies and Research in Biotechnology, Government Science College, Bangalore, Karnataka, India

ABSTRACT

Plant-derived substances have been used since antiquity for therapeutic and other purposes. The present study was undertaken to make a comparative analysis of antimicrobial and antioxidant properties of 8 medicinal plants, which are used in traditional medicine system. Methanolic extracts of Centella asiatica (leaves), Mentha piperita (leaves), Calotropis gigantean (leaves), Vitex nigundo (leaves), Bauhinia racemosus (leaves), Emblica officinalis (fruit), Bauhinia purpurea (leaves), Asperagus racemosus (stem) were tested for anti-microbial effects using the standard Agar Diffusion method. Antioxidant attributes of above extracts were studied by testing their ability to scavenge DPPH and ABTS radicles. Our results indicated that all tested extracts, with the exception of Bauhinia purpurea, were effective against E. Coli, while Candida albicans was only sensitive to Asperagus racemosus. Among tested extracts, Bauhinia racemosus was most potent in exhibiting radical scavenging activity. Our data provides rationale for exploitation of these plants for antimicrobial and antioxidant attributes.

Keywords : ABTS radical, Antibacterial activity, Antifungal activity, Antioxidant activity, DPPH radical, Plant extracts

I. INTRODUCTION

Plants have served as source of molecules with therapeutic properties from time immemorial and form important part of many traditional medicine systems such as Ayurveda, Siddha, Unani, Homeopathy, Naturopathy, Chinese medicine, folk medicine, etc. [1][2]. Molecules obtained from plants can act as lead compounds, which may later help in attributing new therapeutic properties to them leading to drug discovery. Of late molecules obtained from natural compounds are getting more attention because people have realized that modern medicine system, which utilizes pure synthetic organic compounds for drug designing can have their own side effects and naturally obtained molecules are considered comparatively safe [1].

Antioxidant and antimicrobial properties of natural compounds are also among many therapeutic properties widely sought out in the area of phytochemistry. “An imbalance between oxidants and antioxidants in favor of oxidants, potentially leading to damage, is termed ‘oxidative stress’ ” [3]. Oxidants are products that are usually produced due to aerobic metabolism and it increases during the pathophysiological state [3]. Oxidative stress is mainly caused by reactive oxygen species and in vivo, there are both enzymatic and non-enzymatic ways of reducing oxidative stress [4]. Oxidative stress is known to be responsible for the initiation and

advancement of many pathophysiological conditions[5]. Antioxidants are the answer to damaging effects of oxidants because they delay or prevent oxidation of oxidizable substrates. Antioxidants are synthesized in-vivo with the help of enzymes such as reduced glutathione, superoxide dismutase etc., but they can also be substituted exogenously[6][7]. Plants serve as an excellent source of exogenous antioxidants, especially through dietary source [8]. Earlier studies have shown that most of the plants with therapeutic potential also have good antioxidant properties [8][6]. Antimicrobial property in plants is also quite a well-explored region of research. Growing resistance to antibiotics and need for developing molecules with better antimicrobial property has always made researchers look for newer sources for antimicrobial molecules, and plants have served as an excellent source of nutraceuticlas with potent bioactivity [9][10][11]. Many researchers have concentrated on screening antimicrobial properties in plants of some particular region[12][13] and some have concentrated on specific plant products like essential oils or just extract from one particular solvent [14][13]. Nevertheless, there has been a continuous search for molecules with antimicrobial properties in plants.

II. METHODS AND MATERIAL

A. Collection of plant material

The plants Centella asiatica (leaves), Mentha piperita (leaves), Calotropis gigantean (leaves), Vitex nigundo (leaves), Bauhinia racemosus (leaves), Emblica officinalis (fruit), Bauhinia purpurea (leaves), Asperagus racemosus (stem) were collected from Gandhi Krishi Vignana Kendra, Bangalore. The identity of plant materials were confirmed by Prof. Nataraj, Assistant professor in Botany, Government Science College, Bangalore, based on taxonomic characters of specimen.

B. Methanolic extraction of plant material

Collected plant material was shade dried, pulverized into powder and was sieved using 0.5 mm mesh to obtain a uniform fine powder. Thus obtained fine power was stored in air tight containers for all further uses.

For preparation of extract 10 g of powder with 50 ml methanol was taken (1:5 ratio) and this mixture was incubated at room temperature for 24 hours. The extract was obtained after filtration of the mixture using Whatmann filter paper 1. The filtrate was then concentrated using rotatory evaporator until a semisolid extract was obtained. The yield of extract was calculated and is tabulated in table 1.

C. Sample preparation for qualitative phytochemical analysis

1) **Preparation of working solution:** 100mg of each methanolic extracts were dissolved in 1ml of distilled water to get 100mg/mL concentration. 100µL of this extract was dissolved in 400µL of distilled water to get an aqueous extract 5mg/ml. This extract was used to conduct different phytochemical tests.

D. Sample preparation for antimicrobial tests

1) **Preparation of stock solution:** 100mg of methanolic plant extract was weighed and dissolved in 1 mL methanol(100 mg\mL).

Standard antibiotics Ciprofloxacin (100 mg/mL) and Fluconazole (100 mg/mL) were dissolved in methanol and diluted with sterile distilled water to give a final concentration of 1mg/mL.

ii) **Sample preparation for antioxidant assays:** 100 mg/ml of each plant extract was prepared using methanol. This extract was then used to determine anti-oxidant activity.

E. Qualitative analysis of phytoextract

Standard protocols were followed for screening the extracts to test the presence of different phytochemicals. Tests were conducted to screen the presence of carbohydrates, aminoacids, alkaloids, tannins, saponins, flavonoids, terpenoids, steroids and

glycosides. Results of the conducted tests are presented in the table 2.

F. **Anti-microbial activity**

The phytoextracts were tested for the presence of antimicrobial activity using following five test organisms. They are bacteria Bacillus subtilis , Enterobacter aerogenes , Staphylococcus aureus , Escherichia coli and the fungus Candida albicans. MHA(Muller Hinton Agar) media was used for all bacteria and SDA was utilized for fungal growth. Disc diffusion method was utilized here to check the antimicrobial activity of phytoextracts.

1) **Preparation of discs and sterile swabs:** 6 mm disc were punched out of Whatmann filter paper No. 1. The discs and swabs were autoclaved before use.

2) **Preparation of Bacterial inoculums:** Bacillus subtilis, Escherichia coli, Enterobacteria aerogenes, Strephylococcus aureus, culture were grown overnight (16-18 hrs) in nutrient agar plates. A loopful of culture was suspended in 0.85 % saline and the optical density at 620 nm of culture suspension was adjusted to 0.15 (0.5 Mc Farland). The Inoculums was diluted with 2 volumes of MHB to give a final cell density of 105 per ml.

3) **Preparation of fungal Spore suspension:** Candida albicans was grown in Sabouraud dextrose agar (SDA) for 48 – 72 hrs. The fungal spores were harvested with inoculums loop and suspended in 0.85 % saline and the optical density at 620 nm of culture suspension was adjusted to 0.15 (0.5 Mc Farland). The inoculums were diluted with 2 volumes of MHB to give a final cell density of 105 per ml.

4) **Inoculation:** The bacterial cells and fungal spore suspension (100 µL) were inoculated onto the entire surface of a MHA plate and SDA plate with a sterile cotton-tipped swab to form a lawn. The paper disks (6 mm in diameter) impregnated with 10 µl of extracts and allowed to air dry, placed even on the surface of each culture plate using a sterile pair of forceps. Similarly discs impregnated with Ciprofloxacin (5 µg/disc) and Fluconazole (10 µg/disc) were used as positive control for bacterial and fungal culture plates respectively. Discs impregnated with 10 µL methanol served as control. The bacterial plates were incubated at 37°C for 18 – 24 Hrs and fungal plates were incubated at 22°C for 48 – 72 Hours. The plates were observed for zone of inhibition and diameter was measured by a ruler and compared with standard antibiotic.

Anti-microbial activity of the plant extracts were determined by the measurement of inhibition zone. The inhibition zone is calculated using the formula

D=D1-D2/2

D1=Measurement of inhibition zone horizontally.

D2= Measurement of inhibition zone vertically.

D=Zone of inhibition.

All the experiments were conducted in triplicates and the average of 3experimentally determined values are tabulated in table no. 3

G. **Antioxidant activity**

Antioxidant activity of the plant extracts was done using ABTS radical scavenging assay and DPPH assay.

1) ABTS assay - ABTS radical scavenging assay of plant extracts was performed according to [15].

ABTS assay is based on a simple principle of scavenging of light by 2,2'-azinobis-ethyl-benzothiozoline-6-sulphonic acid (ABTS) radicals. The assay was performed as per Auddy.et.al, (2003). ABTS radical cations were produced by reacting (ABTS) (7mM prepared in 125mM phosphate buffered saline Ph 7.4 (PBS)) and Ammonium per sulfate 2.45mM (also prepared with PBS) on incubating the mixture at room temperature in dark for 16 hours. The solution thus obtained was further diluted with PBS to give an absorbance of 1.000. Different concentrations of the test sample and the reference standard (highest volume taken was 50µl) were added to 950 µl of ABTS working solution to give a final volume of 1ml, made up by adding PBS. The absorbance was recorded immediately at 734nm. Gallic acid(100µg/ml) was used as the reference

standard. The percent inhibition was calculated at different concentrations and the IC_{50} values were calculated by Non-linear regression analysis.

2) DPPH Anti-oxidant Assay

DPPH [1, 1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple color and antioxidants reduce DPPH to 1,1-diphenyl-2-picryl hydrazine a colorless compound which can be measured at 510 nm. DPPH solution was prepared by dissolving 1.3 mg DPPH in 1 ml HPLC grade methanol, Gallic acid (5mg/100ml) in methanol was used as reference standard.

DPPH assay was carried out as per [16].In brief, 90 µl of DPPH solution was treated with 180µl of various concentrations of test solution & standard. The different concentrations tested for reference standard were 0.5, 1.0, 1.5, 2.0, 2.5 µg/ml. The reaction mixture is mixed and incubated at 25°C for 15 minutes. The absorbance is measured at 510 nm using Plate reader. A control reaction is carried out without the test sample.

Determination of % Inhibition

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Sample})}{(\text{Control})} \times 100$$

Statistical Analysis

IC_{50} values for DPPH radical scavenging activity of test compounds is derived from a nonlinear regression analysis (curvefit) based on sigmoidal dose response curve (variable) and computed using Graph Pad Prism 5.

III. RESULTS AND DISCUSSION

TABLE 1. YIELD OF METHANOLIC EXTRACT

PLANT	WEIGHT OF EXTRACT
M.piperita	0.76g/10g
C.asiatica	0.3122 g/10g
C.gigantea	1.1833 g/10g
V. nigundo	1.3681 g/10g
E. officinalis	3.7731 g/10g
B.racemosus	2.4097 g/10g
B.purpurea	2.4907 g/10g
A.racemosus	2.9299 g/10g

TABLE 2. Qualitative Analysis of Phytochemicals

TEST	*M. piperita*	*C.asiatica*	*C.gigantea*	*B.racemosus*	*E.officinalis*	*V.negundo*	*B.purpurea*	*A.resemosus*
Carbohydrates	+	+	+	+	+	+	+	+
Amino acids	+	_	+	_	_	_	_	_
Alkaloids	+	+	_	+	_	+	+	_
Tannins	+	+	_	+	_	+	+	_
Saponins	_	_	_	+	_	_	_	_
Flavonoids	_	_	_	+	+	_	_	_
Terpenoids	+	+	+	_	+	_	_	+
Glycosides	+	+	+	+	_	+	+	_
Steroids	+	+	+	+	+	+	+	+

TABLE 3. Antimicrobial Activity of Plants

Plant/Test organsim	*Staphylococcus aureus*	*Bacillus subtilis*	*Enterobacter aerogenes*	*Escherichia coli*	*Candida albicans*
Centella asiatica	1.65cm	N.D.	0.85cm	1.25cm	N.D.
Mentha piperita	N.D.	1.2cm	N.D.	1.04cm	N.D.
Calotropis gigantea	0.6cm	N.D.	N.D.	1.05cm	N.D.
Vitex nigundo	N.D.	1.35cm	1.4cm	1.06cm	N.D.
Bauhinia racemosus	1.1cm	N.D.	1.2cm	0.9cm	N.D.
Emblica officinalis	1.4cm	N.D.	N.D.	1.55cm	N.D.
Bauhinia purpurea	N.D.	N.D.	1.0CM	ND	N.D.
Asperagus racemosus	N.D.	3.25cm	N.D.	1.25cm	2.2cm
Standard	2.8cm	2.8cm	2.8cm	1.4cm	1.1cm

It is evident from the above table that *Centella asiatica, Vitex nigundo, Bauhinia racemosus* have broad spectrum antibacterial activity showing activity against atleast 3 different bacterial strains. *Emblica officinalis* is showing a zone of inhibition bigger than that of standard drug against E. coli. Though majority of the phytoextracts did not show any kind of antifungal activity, *Asparagus racemosus* exhibited two important properties. First of all it is active against two bacterial strains and interestingly it is showing better inhibitory effect than the standard drug in one of the bacterial strain. Second observation is that it is the only plant showing significant antifungal activity among all the plants considered in the present study. It's antifungal activity is better that of standard drug fluconazole.

TABLE 4. ABTS Assay

Plant	IC_{50} Values in µg/ml
Centella asiatica	N.D.
Mentha piperita	147.5
Calotropis gigantea	78.92
Vitex nigundo	81.59
Bauhinia racemosus	71.78
Emblica officinalis	87.12
Bauhinia purpurea	87.67
Asperagus racemosus	N.D.

According to the data obtained using ABTS radical scavenging assay among the eight plants studied *B. racemosus* is showed the best antioxidant acivity and *M.piperita* is showed least antioxidant activity. Radical scavenging activity in case of *C.asiatica* and *Asperagus racemosus* was not detectable since tested concentration did not exhibit 50% inhibition under performed experimental conditions

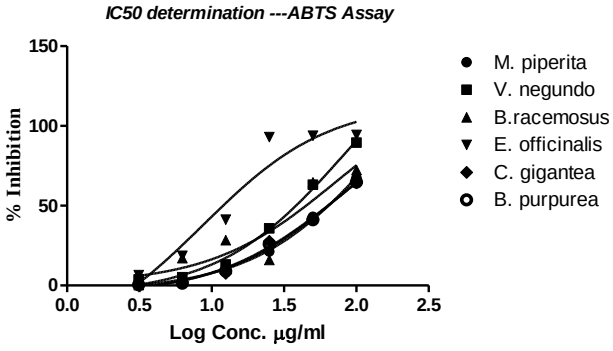

Figure 1

TABLE 5. DPPH Assay

Plant	IC_{50} Values in µg/ml
Centella asiatica	30.64
Mentha piperita	N.D.
Calotropis gigantea	N.D.
Vitex nigundo	46.10
Bauhinia racemosus	19.34
Emblica officinalis	23.56
Bauhinia purpurea	N.D.
Asperagus racemosus	N.D.

According to the data obtained using DPPH assay, among the eight plants studied *B. racemosus* is showing the best antioxidant acivity and *C.asiatica* is showing least antioxidant activity. Radical scavenging activity in case of *M.piperita, C.gigantea, B.purpurea* and *A.racemosus* was not detectable since tested concentration did not exhibit 50% inhibition underperformed experimental conditions.

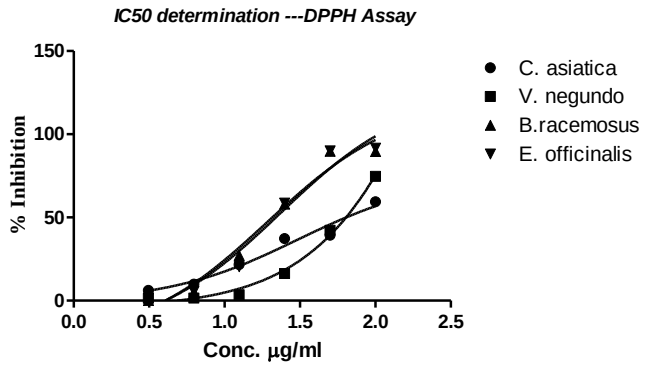

Figure 2

IV.CONCLUSION

Our present work was aimed at screening certain medicinal plants for their antimicrobial and antioxidant properties. From the present study we can conclude that, five among eight plants that were analyzed are potential candidates for our further research in the field of phytochemistry, they are *Centella asiatica, Vitex nigundo, Bauhinia racemosus, Emblica officinalis and Asparagus racemosus. Centella asiatica, Vites nigundo, Bauhinia racemosus* have broad spectrum antibacterial activity, by being active against three different bacterial strains. *Emblica officinalis* is showing a bigger zone of inhibition compared to standard drug which is a notable fact. Though majority of the phytoextracts did not show antifungal activity, *Asparagus racemosus* phytoextract show antibacterial as well as antifungal activity. Infact when tested on *Bacillus subtilis,* and *Candida albicans* extracts from *Asperagus racemosus* is showing bigger zone of inhibition than the standard drugs under our experimental conditions. The results are promising but further research is necessary to come to a conclusion which particular compounds are responsible for their properties.

V. REFERENCES

[1]. S. M. K. Rates, “Plants as source of drugs," Toxicon, vol. 39, no. 5, pp. 603-613, 2001, doi: 10.1016/S0041-0101(00)00154-9.

[2]. A. K. Shakya, “Medicinal plants: Future source of new drugs," Int. J. Herb. Med., vol. 4, no. 4, pp. 59-64, 2016.

[3]. H. Sies, “Oxidative stress: Oxidants and antioxidants," Exp. Physiol., vol. 82, no. 2, pp. 291-295, 1997, doi: 10.1113/expphysiol.1997.sp004024.

[4]. D. M. Kasote, S. S. Katyare, M. V Hegde, and H. Bae, “Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications," vol. 11, 2015, doi: 10.7150/ijbs.12096.

[5]. S. B. Rao, M. Jayanthi, R. Yogeetha, H. Ramakrishnaiah, and J. Nataraj, “Free radical scavenging activity and reducing power of

Gnidia glauca (Fresen.) Gilg ARTICLE INFO ABSTRACT," J. Appl. Pharm. Sci., vol. 3, no. 6, pp. 203-207, 2013, doi: 10.7324/JAPS.2013.3634.

[6]. D. M. Kasote, M. V. Hegde, and S. S. Katyare, "Mitochondrial dysfunction in psychiatric and neurological diseases: Cause(s), consequence(s), and implications of antioxidant therapy," BioFactors, vol. 39, no. 4, pp. 392-406, 2013, doi: 10.1002/biof.1093.

[7]. V. Kamath, A. K. R. Joshi, and P. S. Rajini, "Dimethoate induced biochemical perturbations in rat pancreas and its attenuation by cashew nut skin extract," Pestic. Biochem. Physiol., vol. 90, no. 1, pp. 58-65, 2008, doi: 10.1016/j.pestbp.2007.07.007.

[8]. D. Krishnaiah, R. Sarbatly, and R. Nithyanandam, "A review of the antioxidant potential of medicinal plant species," Food Bioprod. Process., vol. 89, no. 3, pp. 217-233, 2011, doi: 10.1016/j.fbp.2010.04.008.

[9]. T. G. Shaikh S., Lochan R., Kaul P., "Beta lactamase Inhibitors from Indigenous Herbs and Spices.," Res. J. Pharm. Biol. Chem. Sci., vol. 5, no. 2, pp. 275-285, 2014.

[10]. J. L. Ríos and M. C. Recio, "Medicinal plants and antimicrobial activity.," J. Ethnopharmacol., vol. 100, no. 1-2, pp. 80-4, Aug. 2005, doi: 10.1016/j.jep.2005.04.025.

[11]. M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," J. Pharm. Anal., vol. 6, no. 2, pp. 71-79, 2016, doi: 10.1016/j.jpha.2015.11.005.

[12]. D. K. B. Runyoro, M. I. N. Matee, O. D. Ngassapa, C. C. Joseph, and Z. H. Mbwambo, "Screening of Tanzanian medicinal plants for anti-Candida activity," BMC Complement. Altern. Med., vol. 6, pp. 1-10, 2006, doi: 10.1186/1472-6882-6-11.

[13]. U. Mabona, A. Viljoen, E. Shikanga, A. Marston, and S. Van Vuuren, "Antimicrobial activity of southern African medicinal plants with dermatological relevance: From an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound," J. Ethnopharmacol., vol. 148, no. 1, pp. 45-55, 2013, doi: 10.1016/j.jep.2013.03.056.

[14]. F. Nazzaro, F. Fratianni, L. De Martino, R. Coppola, and V. De Feo, "Effect of essential oils on pathogenic bacteria," Pharmaceuticals, vol. 6, no. 12, pp. 1451-1474, 2013, doi: 10.3390/ph6121451.

[15]. B. Auddy et al., "Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases," J. Ethnopharmacol., vol. 84, no. 2-3, pp. 131-138, 2003, doi: 10.1016/S0378-8741(02)00322-7.

[16]. V. Rajkumar, G. Guha, and R. Ashok Kumar, "Antioxidant and anti-neoplastic activities of Picrorhiza kurroa extracts," Food Chem. Toxicol., vol. 49, no. 2, pp. 363-369, 2011.

Cite this article as :

Kiran Kumar D. J., Bhanupriya H. J. , "Comparative Analysis of a few Indian Medicinal Plants for their Antimicrobial and Antioxidant Properties", International Journal of Scientific Research in Science, Engineering and Technology (IJSRSET), Online ISSN : 2394-4099, Print ISSN : 2395-1990, Volume 7 Issue 7, pp. 73-79, January-February 2020. Available at doi : https://doi.org/10.32628/IJSRSET20718

Journal URL : http://ijsrset.com/IJSRSET20718