

Evaluation Of Bioactivity Of Various Indian Medicinal Plants – An In-Vitro Study

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Abstract

Medicinal plants that are native to India and their use in various traditional system of medicine are induced awe- inspiring. The ethnobotany and ubiquitous plants provide a rich resource for natural drug research and development. In the present study, we evaluated the bioactivity of various medicinal plants like *Adathoda vasika*, *Aegle marmelos*, *Baliospermum montanum*, *Citrus limon*, *Clerodendron inerm*, *Euphorbia hirta*, *Ficucs bengalensis*, *Hyptis suaveolens*, *Physalis minima* *Melothira medaraspatisensis* and *Solanum torvum*.. The antimicrobial potentials were observed with the extracts of *Adathoda vasika*, *Ficus bengalensis* and *Solanum torvum* The LD₅₀ value of all the plant extracts were determined against Peripheral blood mononuclear cells (PBMC) and the concentration below LD₅₀ was taken for further study to determine the antioxidant property. The antioxidant activity was highly observed in *Euphorbia hirta*, *Hyptis suaveolens* and *Physalis minima*. The phytochemical analysis of methanolic extracts of all the plants revealed the presence of secondary metabolites.

INTRODUCTION

Infectious diseases are the leading cause of death Worldwide. The problem of microbial resistance and degenerative diseases are growing and the outlook for the use of drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem, for example, to control the use of antibiotics, develop research to better understand the genetic mechanism of resistance and continue studies to develop new drugs, either synthetic or natural (Nascimento et al., 2000).

Oxidation is essential in many living organisms, for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals which is involved in the onset of many diseases such as atherosclerosis, rheumatoid arthritis and cancer as well as in degenerative processes associated with aging (Halliwell et al., 1988). These free radical damage are well protected by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherols and glutathione (Mau et al., 2002). When the mechanism of antioxidant protection becomes unbalanced, deterioration of physiological functions may occur resulting in diseases. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies to reduce oxidative damage (Mohan et al., 2008).

Many infectious and oxidative degenerative diseases have been known to be treated with the herbal remedies throughout the history of mankind. The importance of herbs in the management of human ailments cannot be over emphasized. The plant harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. These herbal remedies give the plant a whole safety and efficiency much superior (Shariff, 2005). Natural products either as standardized plant extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Parekh and Chand, 2006). The cost of production of synthetic drugs is also high and they produce adverse effect compared to plant derived drugs. Hence much attention has been paid recently, to the biologically active compounds derived from plants used in herbal medicine.

MATERIALS AND METHODS

PLANTS USED

Adathoda vasika, *Aegle marmelos*, *Baliospermum montanum*, *Citrus limon*, *Clerodendron inerm*, *Euphorbia hirta*, *Ficucs bengalensis*, *Hyptis suaveolens*, *Physalis minima*, *Melothira medaraspatisensis* and *Solanum torvum*.

EXTRACTION

All the plant leaves were shade dried. The dried leaves were

ground with methanol using mortar and pestle and centrifuged at 5000rpm for 5min to collect supernatant. The supernatant was air dried and the dried material was used for the study.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of all the plant extracts was determined by Agar disc diffusion method (Bauer et al., 1966). Clinical bacterial isolates like E.coli, K.pneumoniae, Enterobacter, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi A, and Staphylococcus aureus were used for the study. For antifungal activity, Candida albicans isolated from the clinical sample was used.

CYTOTOXICITY ASSAY

Isolation of Peripheral blood mononuclear cells (PBMC) (Hudson and Hay, 1989)

Blood was obtained in a sterile heparinized tube. 1:1 dilution of blood with PBS was made. 5ml was overlaid carefully over 2.5 ml of histopaque (HiMedia, India). this was spun at 1600 rpm for 30 minutes. buffy coat was carefully aspirated with sterile pipette. enough Phosphate Buffer Saline (PBS) was added to the PBMC's to make up 5mL and was spin at 1200 rpm for 10 minutes. Supernatant was decanted, and washed once again in PBS. This PBMC was used for cytotoxicity assay.

MTT ASSAY

Cytotoxicity was evaluated against PBMC by MTT assay (Mosmann, 1983) and Lethal Dose 50 (LD₅₀) of every plant extract was determined by %cytotoxicity and by graphical illustration,

Figure 1

$$\% \text{ cytotoxicity} = \frac{\text{Mean OD of test (Plant extract)} \times 100}{\text{Mean OD of control}}$$

The concentration below LD₅₀ was used for the evaluation of antioxidant activity.

FREE RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of all the plant extracts was evaluated by DPPH method (Brant-Williams, 1995).

ANTIOXIDANT ACTIVITY

1 x 10⁴ PBMC/ 100µl was taken in a 96 well sterile tissue culture plate and it was subjected to oxidative stress by addition of 5µl of 30% w/v Hydrogen peroxide. Plant extracts were then added to the wells. Non- enzymatic assays

like GSH (Brant-Williams et al., 1995) and TBARS (Konings and Drijver, 1979) were estimated. Enzymatic methods like catalase (Aebi, 1974), SOD (Poonam, 1984) and GPx (Poonam, 1984) were also determined.

TESTS FOR SECONDARY METABOLITES (EDEOGA ET AL., 2005).

TANNINS

0.5 g of dried extract was taken in a boiling tube and was boiled with 20 ml of water and then filtered. Few drops of 0.1% ferric chloride were added mixed well. Development of brownish green or a blue - black coloration confirms the presence of tannin.

PHLOBATANNINS

1 g of extract was taken in a boiling tube and it was boiled with 2 ml of 1% aqueous hydrochloric acid. This was allowed to stand for some time to develop color. Development of red precipitate is taken as evidence for the presence of phlobatannins.

SAPONIN

2 g of extract was taken in a boiling tube and was boiled with 20 ml of distilled water and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for the development of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion. Formation of emulsion confirms the presence of saponin.

FLAVANOIDS

5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by addition of concentrated sulphuric acid and was allowed to develop color. Development of yellow coloration indicates the presence of flavanoids.

STERIODS

2 ml of acetic anhydrides was added to 0.5 g ethanolic extract. 2 ml of Sulphuric acid was added to that and was allowed to react and to develop colour. The color changes from violet to blue or green confirms the presence of steriods.

TERPENOIDS (SALKOWSKI TEST)

5 ml of the extract was mixed with 2 ml of chloroform in a test tube. Concentrated sulphuric acid (3 mL) was carefully added over that to form a layer. Development of reddish brown color at the interface indicates the presence of

terpenoids.

GLYCOSIDES (KELLER-KILIANI TEST)

5 ml of the extract was treated with 2 ml of glacial acetic acid, followed with one drop of ferric chloride solution in a test tube. 1 ml of concentrated sulphuric acid was overlaid to that. Formation of brown ring at the interface indicates the presence of glycosides.

RESULTS AND DISCUSSION

The present investigation demonstrates the significant antimicrobial activity of plant extracts belonging to different families of the plant kingdom to show the fact that plants are still reservoir of many pharmaceuticals which can be noted and used in treating infectious diseases. Antimicrobial activity of methanolic extract of all the plants is presented in Table 1. Significant antimicrobial activity was found with *Adathoda vasika* and *Ficus bengalensis* against *Pseudomonas aeruginosa* and *Solanum torvum* against *Staphylococcus aureus*. It may be due to the presence of secondary metabolites in plants. The above plants might be containing higher concentration of flavonoids, where flavonoids are found to exhibit antibacterial activity (Mohan et al., 2008). None of the plant extracts were found to possess antifungal activity against *Candida albicans*.

Figure 2

Table 1: Antimicrobial Activity of Plant Extracts

S. no	Plants Used	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Enterobacter</i>	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus aureus</i>	<i>Candida albicans</i>
1	<i>Adathoda vasika</i>	-	-	-	-	+	-	-	-	-
2	<i>Aegle marmelos</i>	-	-	-	-	-	-	-	-	-
3	<i>Baliospermum montanum</i>	-	-	-	-	-	-	-	-	-
4	<i>Citrus limon</i>	-	-	-	-	-	-	-	-	-
5	<i>Clerodendron inermis</i>	-	-	-	-	-	-	-	-	-
6	<i>Euphorbia hirta</i>	-	-	-	-	-	-	-	-	-
7	<i>Ficus bengalensis</i>	-	-	-	-	+	-	-	-	-
8	<i>Hyptis suaveolens</i>	-	-	-	-	-	-	-	-	-
9	<i>Melothria medaraspatisensis</i>	-	-	-	-	-	-	-	-	-
10	<i>Physalis minima</i>	-	-	-	-	-	-	-	-	-
11	<i>Solanum torvum</i>	-	-	-	-	-	-	+	-	-

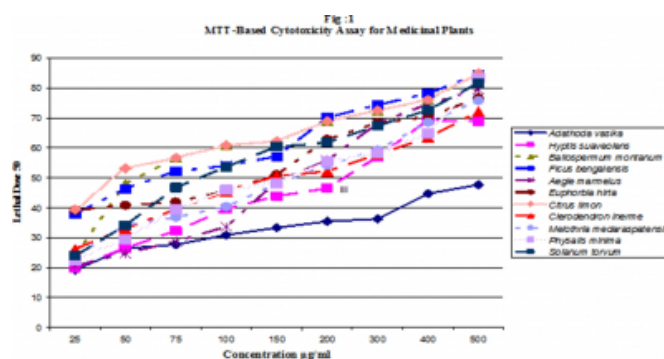
+ showed activity

-- showed resistant

mitochondrial enzyme succinate dehydrogenase and helps in determining the cytotoxicity. The LD₅₀ values of all the plant extracts against Peripheral blood mononuclear cells (PBMC) were determined by MTT assay (Fig 1). *A. vasika* did not show any cytotoxicity on PBMC even at the higher concentration ($\leq 500 \mu\text{g/ml}$). *H. suaveolens*, *E. hirta*, *A. marmelos* and *P. minima* LD₅₀ values was $\leq 140 \mu\text{g/ml}$ on PBMC.

B. montanum exhibited the LD₅₀ value at $\geq 45 \mu\text{g}$. The LD₅₀ value of *C. limon* was found to be $\leq 40 \mu\text{g}$. The LD₅₀ value of *C. inermis* was about $\geq 110 \mu\text{g}$. The LD₅₀ of *F. bengalensis* was found to be around $\leq 55 \mu\text{g}$. *H. suaveolens* expressed the LD₅₀ value around $\leq 200 \mu\text{g}$. The LD₅₀ value of *E. hirta* was $\geq 155 \mu\text{g}$. The LD₅₀ values of *M. medaraspatisensis* and *P. minima* were found to be around $\leq 150 \mu\text{g}$ (Fig.1). The half of the concentration of LD₅₀ concentration of the plant extracts had been selected for the study of antioxidant activity.

Figure 3



This study revealed that all the plant extracts possessed free radical scavenging activity. Among all plant extracts, *Melothria medaraspatisensis* was found to have least free radical scavenging activity (Table 2).

The MTT assay, which evaluates the activity of

Figure 4

Table 2: Free Radical Scavenging Activity (DPPH method)

S.No	Plants Used	DPPH % ± SE
1	<i>Adathoda vasika</i>	48.45 ±1.56
2	<i>Aegle marmelos</i>	29.83 ±1.40
3	<i>Baliospermum montanum</i>	12.51± 1.11
4	<i>Citrus limon</i>	6.76 ±1.07
5	<i>Clerodendron inerm</i>	46.85± 0.79
6	<i>Euphorbia hirta</i>	19.43±0.76
7	<i>Ficus bengalensis</i>	32.68±1.29
8	<i>Hyptis suaveolens</i>	35.11±1.41
9	<i>Melothria medaraspatisensis</i>	3.06± 1.34
10	<i>Physalis minima</i>	29.55± 1.53
11	<i>Solanum torvum</i>	29.8 ± 0.89

The improper balance between reactive oxygen intermediates and antioxidants defense results in 'oxidative stress' and it cause damage to the cells. Cellular antioxidant enzymes such as catalase, SOD and GPx normally challenge oxidative stress. Antioxidant activity of all plant extracts were represented in Table 3.

Figure 5

Table 3: Antioxidant activity of Plant Extracts

S.No	Plants Used	TBARS ^a MDA mmol/ml	GSH ^a	Catalase ^a	SOD ^a	GPx ^a
1	<i>Adathoda vasika</i>	0.563 ± 0.55	0.17 ±0.01	0.41±0.02	0.28 ±0.04	0.29 ±0.19
2	<i>Aegle marmelos</i>	0.43 ±0.56	0.71 ±0.24	0.33±0.12	0.23 ±0.18	0.29± 0.10
3	<i>Baliospermum montanum</i>	0.46 ± 0.58	1.06±0.008	0.27 ±0.05	0.19± 0.2	0.3 ± 0.17
4	<i>Citrus limon</i>	0.19 ±0.36	0.12 ±0.12	0.25 ±0.18	0.27 ±0.13	0.27± 0.18
5	<i>Clerodendron inerm</i>	0.15 ±0.35	0.26 ±0.13	0.21±0.004	0.14 ±0.13	0.31± 0.20
6	<i>Euphorbia hirta</i>	0.03 ±0.18	0.62 ±0.05	0.36±0.03	0.21 ±0.12	0.27± 0.19
7	<i>Ficus bengalensis</i>	0.41 ±0.46	1.06±0.08	0.28±0.002	0.28 ±0.02	0.28± 0.16
8	<i>Hyptis suaveolens</i>	0.04±0.22	0.05±0.06	0.35±0.004	0.25±0.15	0.26±0.19
9	<i>Melothria medaraspatisensis</i>	0.13 ±0.36	0.2 ±0.29	0.24±0.05	0.22 ±0.12	0.28± 0.13
10	<i>Physalis minima</i>	0.05 ±0.2	0.63 ±0.03	0.37 ±0.03	0.21 ±0.01	0.29± 0.17
11	<i>Solanum torvum</i>	0.19 ±0.12	0.14 ±0.17	0.28 ±0.38	0.28 ±0.24	0.28 ± 0.2

C.inerme, E.hirta and H.suaveolens were found to reduce the GSH level than the control. TBARS of stress induced PBMC was reduced in the presence of E.hirta and H.suaveolens. All the plant extracts were found to reduce GPx activity. Among all H.suaveolens found to have greater activity. None of the plant extracts were found to reduce catalase activity on PBMC. H.suaveolens and P.minima alone were found to reduce the SOD activity. All plant extracts were found to contain polyphenols, E.hirta, H.suaveolens and P.minima might be containing polyphenols in higher concentrations, where as polyphenols are found to possess

antioxidant activity (Yoshida et al., 1999). They have their redox property which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides (Mohan et al., 2008).

Figure 6

Table 4: Secondary metabolites in Plant extracts

S. No	Plants Used	Tannin	Phloba tannin	Saponin	Flavanoids	Steroids	Terpe- noids	Poly- phenol	Glyco- side
1	<i>Adathoda vasika</i>	+	-	-	+	-	+	+	-
2	<i>Aegle marmelos</i>	+	-	+	+	-	+	+	+
3	<i>Baliospermum montanum</i>	-	-	-	+	-	+	+	+
4	<i>Citrus limon</i>	-	-	-	+	-	+	+	-
5	<i>Clerodendron inerm</i>	-	-	+	+	-	-	+	-
6	<i>Euphorbia hirta</i>	-	-	-	+	-	-	+	-
7	<i>Ficus bengalensis</i>	-	-	-	+	-	+	+	+
8	<i>Hyptis suaveolens</i>	+	-	-	+	-	-	+	-
9	<i>Melothria medaraspatisensis</i>	+	-	-	+	-	-	+	-
10	<i>Physalis minima</i>	+	-	-	+	-	-	+	-
11	<i>Solanum torvum</i>	+	-	+	+	-	-	+	-

+ = presence

-- absence

CONCLUSION

The methanolic extract of *Ficus bengalensis*, *Adathoda vasika* and *Solanum torvum* were found to have antimicrobial activity. The plant extracts showed cytotoxic activity against PBMC only at highest concentration ($\leq 200\mu\text{g}$). *Hyptis suaveolens*, *Euphorbia hirta* and *Physalis minima* were found to have antioxidant effect on PBMC. In future, these plants could represent antioxidant agents, which provide prophylaxis against various diseases related to oxidative stress. The phytochemical screening indicated the presence of compounds which are mainly responsible for the remarkable antioxidant and antimicrobial activity. To conclude the obtained result could form a good basis for selections of plant species for further investigation in the potential discovery of new valuable bioactive compounds.

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