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SCREENING OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY IN METHANOL EXTRACT OF TODDALIA ASIATICA L

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ABSTRACT
The medicinal plant Toddalia asiatica L. belongs to the family Rutacee and commonly called as JungliMirachi. Toddalia asiatica grows in tropical and sub-tropical regions of India and used for variety of purposes in traditional medicine. Toddalia asiatica were extracted with solvent methanol and screened. The aim of the current study was undertaken in order to evaluate the antioxidant activity and antimicrobial activity of Toddali aasiatica leaf by using solvent extract methanol. Further In vitro antioxidant activities such as DPPH, Fe3+, ABTS radical scavenging assay were carried out. Thus, the work clearly indicates that the methanol leaf extract of was found to be highly potential in antioxidant as well as antimicrobial activity.

KEYWORDS: Antioxidant, DPPH, Fe3+, ABTS, Disc diffusion and Antimicrobial**.**

INTRODUCTION

India has a rich culture of medicinal herbs and spices, human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982 and Hill, 1989). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu *et al.*, 1999, Idu *et al*., 2007 and Mann *et al*., 2008).

Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai & Roy, 2004 and Tapsell *et al.*, 2006). Even with the advent of modern or allopathic medicine, (Balick and Cox, 1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

FREE RADICALS

An atom or group of atoms with one or more unshared electrons, which may enter into chemical bond formation, is called a free radical. The same group in a molecule is called a radical for example, the methyl radical in methyl cyanide or other molecules (Koppenol WH, 2000).

ANTIOXIDANTS

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). Flavonoids may help provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks.

Medicinal plants continue to generate interest in pharmacological research and development of new anticancer agent. Approximately 64% of drugs used in cancer chemotherapy are from plants materials with different modes of action. Hartwell has estimated the use of over 3000 plant species in the treatment of cancer (Hartwell, 1982).

Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. For example, some studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer, compared with chemotherapy or recent hormonal treatments. (Pezzuto, 1997 and Wu *et al.*, 2002).

Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. These compound such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer properties are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property.

MATERIALS AND METHODS

a. Plant collection

Fresh leaves of *Toddalia asaitic*a were been collected from the regions of thiruvanamalai hills. The leaves were carefully washed with tap water followed by rinsing in distilled water and air-dried at room temperature for few hours. Then leaves were separated and taken to separate clean place and dried at room temperature for one week. Then they were ground into fine powder and sieved through fine mesh, finally stored in cool and dry place in a clean air-tight container

b. Extraction of leaf extract by direct method

Extraction of leaf powder with methanol was performed by direct method of extraction after the method of (Eloff, 1998).

c. Cleaning of glassware

All the glassware (Borosil and Corning) were soaked in cleaning solution for few hours. Then the glassware were been washed thoroughly with tap water, followed by detergent solution and finally rinsed with distilled water. The cleaned glassware were dried in hot air oven at 60^{o} C for few hours and stored.

d. Cleaning solution (Mahadevan and Sridhar, 1996)

Potassium dichromate - 60 g
Concentrated sulphuric acid - 60 mL
Distilled water - 1000mL

e. Sterilization

Dried glassware and culture media were sterilized in an autoclave at 121^{o}C temperature for 15 min at 15 lb/sq inch pressure.

f. Screening of crude compounds for Antioxidant studies

i) Free Radical Scavenging activity by DPPH assay

The antioxidant activity was determined by DPPH scavenging assay (Khalaf *et al.* 2008). Various concentrations (200µg, 400µg, 600µg, 800µg and 1000µg/ml) of the crude extract was been pipetted out in clean test tubes. Freshly prepared DPPH (1,1-Diphenyl-2-picryl hydrazyl) solution (2ml) was added to each tube and the samples were incubated in dark at 37°C for 20 min and read at 517 nm. The data were expressed as the percent decrease in the absorbance compared to the control. Ascorbic acid was used as reference compound. The percentage inhibition of radical scavenging activity was calculated.

%Radical scavenging potential = [(Control OD-Sample OD)/Control OD] X100.

Based on the screening results, one extract possessing maximum activity was selected for other antioxidant studies.

ii) Ferric (Fe^{3+}) reducing power assay

The crude extract was taken in various concentrations (300µg, 350µg, 400µg, 450µg and 500µg/ml) and was mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of potassium ferricyanide (1%), and incubated at 50°C for 30minutes. Then, 2.5ml of trichloroacetic acid (10% v/v) was added to the mixture and then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5ml of upper layer solution was mixed with 2.5ml of distilled water and 0.5ml $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm (Makari *et al*., 2008 and Hennebelle *et al*., 2008). EDTA served as standard.

%Ferric reducing potential = [(Sample OD-Control OD)/Sample OD] X100.

iii) ABTS assay

This assay was performed according to the method of (Arnao *et al*., 2001). The stock solutions included 7.4 mm ABTS solution and 2.6 mm potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 1.170.02 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Test sample (200µL of varying concentration) were allowed to react with 2850 µL of the ABTS solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer.

g) Antibacterial activity

i) Collection of Microbes

Human pathogenic organisms (bacteria) were isolated from clinical samples obtained from Department of Microbiology. The organisms are identified and confirmed by clinical microbiologist. The organisms under study were Staphylococcus aureus, Micrococcus luteus, Escherichia coli and Proteus vulgaris.

ii) Agar Well diffusion assay (Eloff, 1998)

Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 hours grown bacterial pathogens (four) were swabbed on nutrient agar plates. Then, the stock crude extract (Ethyl acetate) individually (10mg/ml) was prepared. Varying concentration (250µg, 500µg, 750µg and 1000µg) was loaded in the wells made using cork borer. Tetracycline was used as standard. The plates were then incubated at 37ºC for 24hours. After incubation the inhibition diameter was measured.

RESULT AND DISCUSSION

These compounds present in a variety of medicinal plants have significant application against human. They are an important source of practical and inexpensive new drugs. Oxidative stress has been implicated in the pathology of many diseases such as inflammatory conditions, cancer, diabetes and the aging. Pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan *et al*., 2004). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity in most of the medicinal plants (Mantle *et al*, 2000). Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores (Dhar *et al*, 1979).

DPPH radical scavenging activity

The scavenging activity of free radicals showed that the data by reducing the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and this capacity increases with increasing concentration as reported. The maximum percentage of DPPH radical scavenging activity was75.11%at 120 µg/mL concentration as shown in (Figure 1). Then it was compared with the standard ascorbic acid (70.95±4.96/12 µg/mL) and the IC_{50} of DPPH radical scavenging activity was 105.78µg/mL concentration. The scavenging ability of the methanolic extract of tubers of *T. asiatica (L.) Lam. Leaves* may be due to its bio compositions such as phenolic acids and flavonoid. The radical scavenging activities of the extracts were determined by using DPPH a stable free radical at 517 nm. 1,1 -diphenyl-2-picrylhydrazyl is a nitrogen-centered free radical, color of which changes from violet to yellow as shown in (Figure 2) on reduction by donation of H or e^- by the methanolic extract of tubers of *T. asiatica (L.) Lam Leaves*.

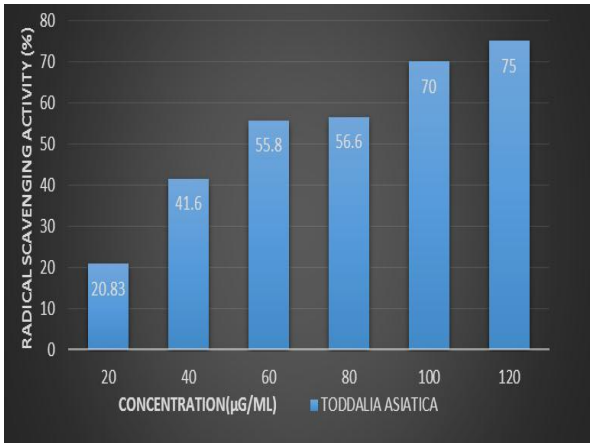


Figure-1: DPPH radical scavenging activity of the methanolic extract

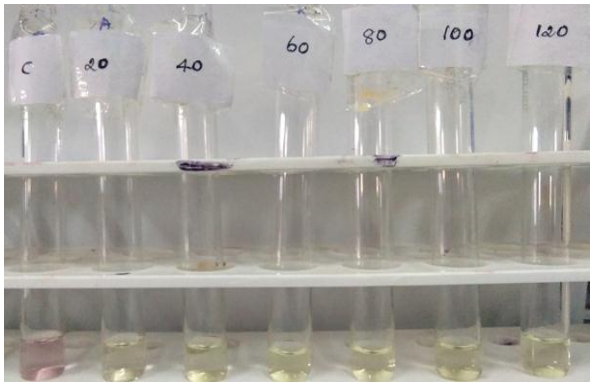


Figure-2: compared with the standard ascorbic acid at 517 nm

ABTS●+ radical scavenging activity

In the total antioxidant activity, ABTS●+ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or trolox, preformed cation radical gets reduced and the remaining radical cation concentration after reaction with antioxidant compound was then quantified. The maximum percentage of ABTS•+ radical cation scavenging activity was 64.91±5.55% at 120 µg/mL concentration as shown in (Figure 3).

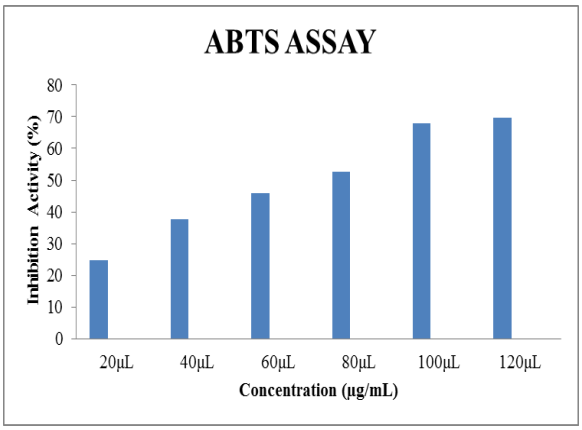


Figure-3: ABTS activity of the methanolic extract of T. asiatica (L.)

Fe^{3+} reducing power assay

The antioxidant activity of *toddalia assiatica* extract was calculated according to the (Makari *et al*., 2008 and Hennebelle *et al*., 2008). The inhibition in reducing power assay as shown in "Figure 4", denotes the yellow color of the test solution changes to various shades of green and blue depends upon reducing power of each compound. The antioxidant activity of Fe^{3+} reducing power assay was 44.06% for methanol extract of *toddalia assiatica* as shown in "Figure 5". At 500µg/ml concentration the maximum activity was observed.

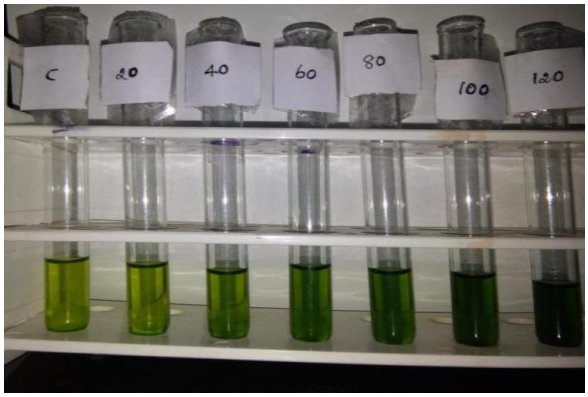


Figure: 4 The inhibition in reducing power assay

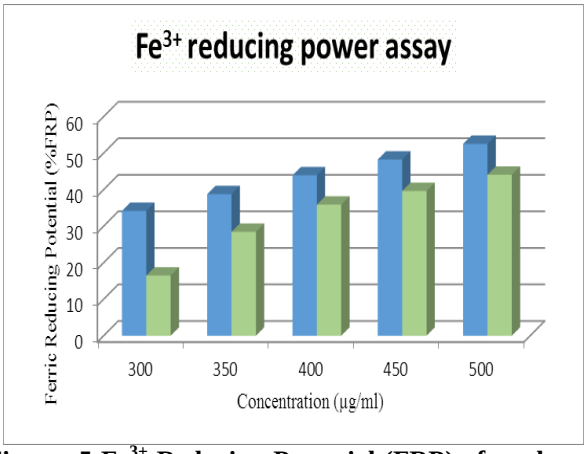


Figure: 5 Fe^{3+} Reducing Potential (FRP) of methanol extract

Antibacterial activity

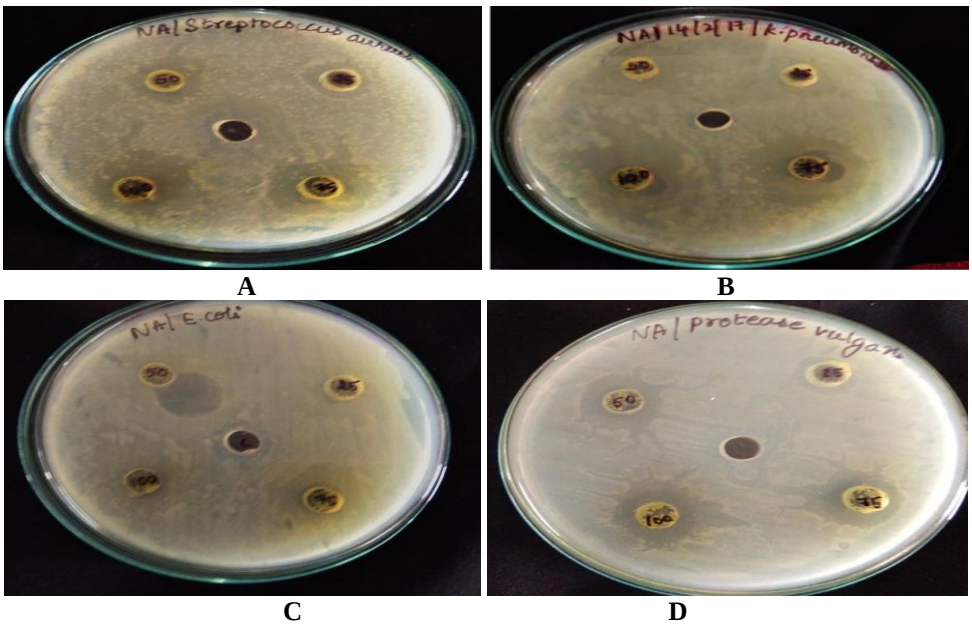
Screening of crude extract for antibacterial activity

The inhibitory activity for the methanol extract was observed after 24hours of incubation. The zone of inhibition was measured using zone scale and is represented in Table 1.

Table: 1 Zone of inhibition measured in mm of methanol extract.

S.No	Bacterial pathogens	Zone of inhibition(mm)				Standard
		25µg	50µg	75µg	100µg	
1	*Streptococcus aurues* (a)	14mm	17mm	20mm	21mm	27mm
2	*Micrococcus luteus* (b)	15mm	17mm	21mm	23mm	24mm
3	*E.coli* (c)	15mm	17mm	18mm	20mm	23mm
4	*Proteus vulgaris* (d)	12mm	17mm	18mm	20mm	23mm

The antibacterial activity of *Toddalia asiatica* was found to be higher against *Micrococcus luteus* inhibiting zone of 23mm at concentration of 1000µg/ml; whereas, the inhibitory effect was been observed for *Micrococcus luteus* (23mm) *E.coli* (20mm) and *Staphylococcus aureus* (21mm).



A **B**

C **D**

Figure-6 A: Zone of inhibition in *Streptococcus aurues* (a)

Figure-6 B: Zone of inhibition in *Micrococcus luteus* (b)
Figure-6 C: Zone of inhibition in *Escherichia coli* (c)
Figure-6 D: Zone of inhibition in *Proteus vulgaris* (d)

CONCLUSION

The present study of the phytochemical screening, showed that, these plant could be a potential source for natural antioxidants. It has been reported that most of the active principles are present.

If these plants are examined for further biological studies, it could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions.

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