Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 124 - 130

Research Article **Biomaterial Sciences**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOLIC EXTRACT OF *TECOMA STANS* FLOWERS

RAJAMURUGAN R[1*], THIRUNAVUKKARASU C[2], SAKTHIVEL V[1], SIVASHANMUGAM M[3] AND RAGHAVAN C M[3]

[1]Department of Biochemistry, M.G.R. College, Hosur-635109 Tamilnadu, India
[2]Department of Biochemistry and Molecular Biology, School of Lifesciences, Pondicherry University, Puducherry-605014
[3]Department of Microbiology, M.G.R. College, Hosur-635109 Tamilnadu, India

ABSTRACT

The aim of the present study was to screen phytochemicals, antioxidant and antimicrobial activities of the extract from *Tecoma stans* flowers. The ethanolic extract of *T. stans* flowers demonstrated antioxidant activity in DPPH radical and β-carotene-bleaching models. The extract exhibited a dose dependent reductive ability. The ethanolic extract of flowers of this species showed strong antimicrobial activity also and was effective against tested bacteria (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae*) and fungi (*Penicillium* sp.). The extract was not effective against *Aspergillus flavus, Cladosporium* sp. and *Botrytis* sp. The phytochemical screening revealed the presence of tannins, flavonoids, phenols, alkaloids, steroids, cardiac glycosides and saponins in the flower extract. The present findings provide scientific evidence to support the traditional use of *Tecoma stans* and also indicate that the flowers of this species are a promising potential for the development of antioxidant and antimicrobial agents.

KEYWORDS: ***Tecoma stans*** **flowers, Phytochemicals, Antioxidant activity, Antimicrobial activity,**

RAJAMURUGAN R
Department of Biochemistry, M.G.R. College, Hosur-635109 Tamilnadu, India

INTRODUCTION

Plants have been used in traditional medicine since prehistoric period and play a significant role to heal human diseases and disorders[1]. According to World Health Organization (WHO), more than 80% of the world's population rely on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno pharmacological information is generally considered as an effective approach in the discovery of new antioxidant and ant-infective agents from higher plants[2]**.** The preservative effect of many plant species suggests the presence of anti-oxidative and antimicrobial constituents in their parts. Many medicinal plants contain large amounts of antioxidants other than vitamin C and carotenoids[3]**.** In recent years, in order to discover novel antioxidant and antimicrobial drugs, screening of plants has been accelerated[4]**.** *Tecoma stans* (L) Juss. Ex Kunth (Bignoniaceae) known as yellow elder is an erect shrub or small tree. The plant has been used for a variety of purposes in herbal medicine, treating diabetes and digestive problems[5]**.** In line with this, the present investigation was undertaken to study the phytochemical compounds and antioxidant and antimicrobial activities of ethanolic extract of the flowers of this species.

MATERIALS AND METHODS

Extraction of plant material

The plant material (*Tecoma stan*s) was collected locally. Authentication of the plant material was made by Dr.M.Shivashanmugam**,** Asst professor in Botany, M.G.R College, Hosur. Flowers were detached and dried in shade. About 100gms of dried flower were ground to powder and exhaustively extracted with 600 ml ethanol using soxhlet apparatus and the extract was concentrated under reduced pressure and then stored in an air tight container for further study.

Phytochemical screening

Phytochemical screening was performed using standard procedures [6, 7].

Evaluation of antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of *Tecoma stans* flower extract was measured by a decrease in the absorbance of an ethanolic solution of DPPH[8]. Different concentration of extract (10-100µg/ml) was added to 2 ml of freshly prepared DPPH. The measurement was performed in triplicates. After incubation for about 30 min at room temperature in the dark, the absorbance was measured at 520 nm using a spectrophotometer (RAYLEIGH). Radical scavenging activity (%) was calculated using the following formula:-

$$\text{DPPH radical scavenging (\%)} = \frac{\text{OD of Control} - \text{OD of Sample}}{\text{OD of Control}} \times 100$$

β –carotene bleaching assay

A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1ml of chloroform and 25µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen was added with vigorous shaking. Aliquots (4.8) of this emulsion were transferred into different tubes containing 0.2ml of different concentrations of the extract, and were incubated for 2 hours at 50 C. A blank devoid of β-carotene, was prepared for background subtraction[9].

Reducing power assay

The reducing power of *Tecoma stans* was determined as per Oyaizu method[10]. Different concentrations of extract (10-100µg/ ml) in 1ml of ethanol were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium

ferrocyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. To a portion(2.5ml) of the reaction mixture, trichloroacetic acid (10%) was added, which was then centrifuged at 3000 rpm for 10 minute upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml,0.1%) and the absorbance was measured at700 nm.

Evaluation of antimicrobial activity

Microbial cultures

The test microbial cultures such as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae* and fungal cultures of *Penicillium* sp.*, Aspergillus flavus, Cladosporium* sp.*,* and *Botrytis* sp. were obtained from Post Graduate and Research Department of Microbiology, M.G.R College, Hosur. Test organisms were subcultured periodically and maintained on their respective growth media for further study.

Screening of antimicrobial activity

The agar-well diffusion method[11] was employed to determine the antimicrobial activities of *Tecoma stans* flower extract against some bacterial sp. (*Escherichia coli, Bacillus subtilius, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus*) and fungal species (*Penicillium* sp. *Aspergillus flavus, Cladosporium* sp.*,* and *Botrytis* sp.) The bacterial cultures were inoculated into the Muller Hinton agar (and incubated at 37 ◦C). Fungal cultures were inoculated into Sabouraud dextrose agar and incubated at 28 ◦C. Approximately, 10ml of sterile Muller Hinton agar (for bacteria) and Sarbourd dextrose agar (for fungi) were poured into sterile culture plates and allowed to set wells of about 8 mm in diameter which were punched on the plates. About 25-100µg/ml of the extract was dispensed into the wells and the plates were incubated at 37°C (for bacteria) and 28°C (for fungi) and observed after 24 h for bacteria and 4 days for fungi.

Statistical analysis

Results are expressed as the mean ± S.D. of three independent experiments (n=3). Student's *t*-test was used for statistical analysis; *P* values > 0.05 were considered to be significant. Linear regression analysis was done for calculating IC_{50} and EC_{50} using Graph Pad prism statistical software.

RESULTS & DISCUSSION

Phytochemical screening

Table 1
Phytochemical screening of Tecoma stans flower extract

Phytochemicals	Flowers of Tecoma stans
Terpenoids	+
Flavonoids	+
Tannins	+
Alkaloids	+
Cardiac Glycosides	+
Steroids	+
Phenols	+
Saponins	+

+ represent present

The medicinal properties of the plants could be credited to the presence of one or more of the active constituents of the plants[12]. Plant phenolic compounds[13] flavonoids[14, 15, 16, 17] alkaloids[18] tannins[13] Saponins[20] from plant extracts and plant based steroids[19] have been found to possess potent antioxidant and antimicrobial properties[13, 14, 15, 16, 17]. The percentage yield of *T. stans* flower was found to be 4.85%. The extract was screened qualitatively for phytochemicals. The results of the preliminary phytochemical screening

revealed the presence terpenoids, alkaloids, flavonoids and steroids (Table 1). cardiac glycosides, tannins, phenols,

Antioxidant activity

Figure 1
DPPH radical scavenging activity of Tecoma stans flower extract

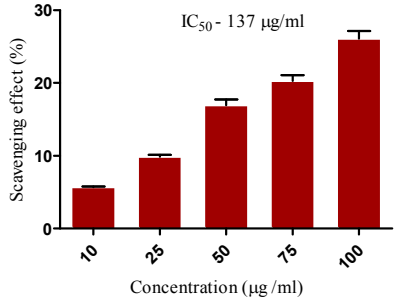

Fig. 1. Scavenging activity (%) on DPPH radicals of Tecoma stans extract. Each value is expressed as mean ± standard deviation (n = 3).

Figure 2
Antioxidant activity of Tecoma stans extract (β-carotene bleaching)

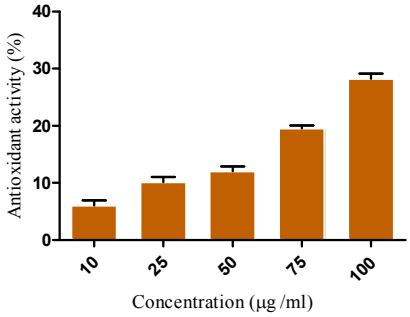

Fig. 2. Antioxidant activity (%) of Tecoma stans extract by β-carotene bleaching method. Each value is expressed as mean ± standard deviation (n = 3).

Figure 3
Reducing power of Tecoma stans extract

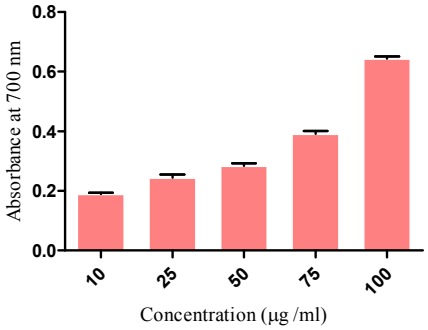

Fig. 3. Reducing power of Tecoma stans extract (higher absorbance indicates higher reducing power). Each value is expressed as mean ± standard deviation (n = 3).

Due to risk of adverse effects encountered with the use of synthetic antioxidants, medicinal plants may offer an alternative source for antioxidants with significant activities against oxidative stress. In this study, antioxidant activity of *T. stans* extract was studied using three *in vitro* methods namely DPPH, β-carotene-bleaching and reducing power assay. The extract showed a dose dependent antioxidant activity in all the three models. The DPPH radical scavenging activity values of *T. stans* extract are presented in Fig. 1; results are expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. From the analysis it is concluded that the scavenging effects of the flower extract *T. stans* on DPPH radicals increased with the increase of concentration. The IC_{50} value is a widely used parameter to measure the free radical scavenging activity. A decrease by 50% of the initial radical concentration is defined as the IC_{50}. A lower IC_{50} value indicates a higher antioxidant activity[21]. In this study, IC_{50} value (µg/ml) of the extract was determined for DPPH and was found to be 137 µg/ml (r^2 0.8586). Fig. 2 shows the antioxidant activity of flower extract *T. stans* as measured by the bleaching of β-carotene. The antioxidant activity of carotenoids is based on the radical adducts of carotenoids with free radicals from linoleic acid. The linoleic acid free radical acts on the highly unsaturated β-carotene models. The presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system[22]. Accordingly, the absorbance decreases rapidly in samples without antioxidant. Whereas, in the presence of antioxidants, samples retain their colour, and thus absorbance, for a longer time. In our study, the results of β-carotene-bleaching assay showed that antioxidant activity of *T. stans* extract increased with the increasing of its concentration with an EC_{50} value of 131 µg/ml (r^2 0.8907). It is probable that the antioxidative components in the *T. stans* extract can reduce the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system. The reducing capacity of a compound may serve as a significant indicator of its potent antioxidant activity. In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. Antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, it can be monitored the Fe^{2+}concentration; a higher absorbance at 700 nm indicates the higher reducing power. It was also reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[23]. In this study the reducing power of *T. stans* was increased with the increase of the dosage ($r^2$0.9562). Fig. 3 shows the reducing power of *T. stans* extract as a function of its concentration.

Antimicrobial activity

Table 2
Antimicrobial activity of Tecoma stans flower extract

Con (µg/ml)	Zone of growth inhibition (mm)					
	Pr.mirablis	*S.aureus*	*E.coli*	*K.pneumoniae*	*B.subtilis*	*Penicillium* sp.
25	15	0	19	18	19	18
50	16	12	19	19	20	21
75	18	13	20	19	21	23
100	19	14	23	21	21	25

In recent years, the search for phytochemicals possessing antimicrobial properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes. In this study, the antimicrobial activities of the ethanol extract of *Tecoma stans* flower gave different zones of inhibition on the organisms tested (Table 2). The extract exhibited strong antibacterial activity against all the five bacterial strains used with diameter of inhibition zone values between 15 and 23 mm (Table – 2). The maximum inhibitory effect was recorded for *E. coli*, 23 mm; followed by *K. pneumoniae*, 21 mm: *B. Subtilis,* 21 mm; *P. mirabilis,* 19 mm and *S. aureus*, 14 mm. Among the fungi tested, the ethanolic extract of *Tecoma stans* was effective only against *Penicillium* sp with diameter of inhibition zone of 25 mm and no activity was observed against *Aspergillus flavus, Cladosporium* sp*,* and *Botrytis* sp*.* The present investigation has shown that the ethanol extract of *Tecoma stans* flowers have active phytochemicals which are able to inhibit the growth of pathogenic bacteria and fungi.

CONCLUSION

From the results of the present study, it could be concluded that *Tecoms stans* flower possesses active pharmacological principles that contribute to free radicals scavenging and antimicrobial properties. The present investigation also suggests that flower of *T. stans* may be utilized as effective and safe natural source for antioxidant and antimicrobial agents. Further*, T. stans* seems to hold great potential for in-depth investigation for various biological activities and the date obtained through this work may be useful in developing new formulations with more therapeutic value.

REFERENCES

1. Chandarana H, Baluja S, and Chanda S. V. Comparison of antibacterial activities of selected species of Zingiberaceeae family and some synthetic compounds. Turk J Biol, 29: 83-97, (2005)
2. Duraipandiyan, V., Ayyanar, and M.,Ignacimuthu, S. Antibacterial Activity of Some *Ethanomedicinal* Plants used by pailyar Tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine, 635, (2006)
3. Jaleel CA, Gopi R, Manivannan P, Kishorekumar A, Sankar B, and Panneerselvam R. Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*. Comptes Rendus Biologies 330 (9): 674-683, (2007)
4. Jack and Okorosaye-Orubite. Phytochemical analysis and antimicrobial activity of the leaves of fleabane (Conyza sumatrensis). J.Appl.Sci.Environ.Manage, 21(4): 63-65, (2008)
5. Senthilkumar, C.S., Sureshkumar, M. and Pandian, M.R. *In vitro* antibacterial activity of crude leaf extracts from *Tecoma stans* (L) Juss. ex Kunth, *Colues forskohlii* and *Pogostemon patchouli* against human pathogenic bacteria. International Journal of Pharma Tech Research, 2(1): 438-442, (2010)
6. Sofowora A. Medicinal Plants and Traditional Medicine in Africa.2nd edn., Spectrum Books Ltd., Ibadan, Nigeria, (2006) pp 151-153, 209-214.
7. Trease G E, and Evans W C. Pharmacognosy. 15th ed. Saunders, London. (2002) pp. 53-336.
8. Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, and Morelli I. Antioxidant principles from *Bauhinia tarapotensis*. J Nat Prod. 64: 892–895 (2001).
9. Tepe B, Sokmen M, Soken A, Daferera D, and Pollission M. Antimicrobial aand antioxidant activity of the essential oil of various extracts of *Cyclotrichium origanifollium* (Labill.)Manden, Scheng J. Food Eng, 69: 335-342, (2004).

10. Oyaizu M.Studies on products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-314, (1986).
11. Collins.C.H, Lynes P M and Grange J M. Microbiological methods, 7th edn. Butterwort-Heinemann Ltd. Britain, (1995) pp 175-190
12. Egwaikhide P A and Gimba C E. Analysis of the phytochemical content and antimicrobial activity of *Plectranthus glandulosis* whole plant. Middle-East Journal of Scientific research, 2(3-4): 135-138, (2007).
13. Kaur, G.J. and Arora, D.S., Antibacterial and phytochemical screening of *Anethum graveolens, Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complement and Alternate Medicine, 9: 30, (2009).
14. Lin, Y., Shi, R., Wang, X. and Shen, H.M. Luteolin, a flavonoid with potential for cancer prevention and therapy. Cure Cancer Drug Targets, 8:. 634-46, (2008).
15. Lopez-Lazaro, M. Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem , 9: 31-59, (2009).
16. Yoshida, T, Konishi, M, Horinaka M, Yasuda T, Goda, A.E, Taniguchi H, Yano K, Wakada M. and Sakai T. Kaempferol sensitizes colon cancer cells to TRAIL-induced apoptosis. Biochem and Biophys Res Comm, , 375: 129-133, (2008).
17. Amarlal S, Mira L, Nogueira J.M, de Silva A.P, and Florencio M.H. Plant extracts with anti-inflammatory properties-a new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships, Bioorg. Med. Chem, 17(5): 1876-1883, (2009).
18. Erdemoglu N, Sozkanm S and Tosun F. Alkaloid profile and antimicrobial activity of *Lupinus angustifolius* L. alkaloid extract. Phytochemistry Reviews, 6(1): 197-201, (2007).
19. Shihabudeen M.S, Priscilla H.D and Kavitha T. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharma Sciences and Research, 1(10): 430-434, (2010).
20. Mandal, P., Babu, S.S.P. and Mandal, N.C., Antimicrobial activity of saponins from *Acacia auriculiformis*. Fitoterepia, 76(5): 462-465, (2005).
21. Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem. 100: 1409–1418 (2007).
22. Jayaprakasha G. K., Singh R. P, and Sakariah K. K. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chemistry, 73: 285–290, (2001).
23. Shimada K., Fujikawa K., Yahara K., and Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agriculture and Food Chemistry, 40: 945–948, (1992).