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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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Evaluation of antioxidant and antimicrobial activities of methanolic extract of *Asparagus racemosus*Willd. roots and *Clitoria ternatea* Linn flowers

REETA TRIPATHI[1*], SHAIL BALA SINGH BAGHEL[2], SHWETA HINGWASIYA[3] AND REENA UPADHYAY[4]

[1]*Department of Chemistry, Barkatullaha University, Bhopal-462026, Madhya Pradesh*
[2]*Sarojini Naidu Govt Girls P. G. College Bhopal-462016, Madhya Pradesh*
[3]*Government P.G. College, Narsinghgarh-486002, Madhya Pradesh*
[4]*Barkatullaha University, Bhopal-462026, Madhya Pradesh*

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Plants are used to treat various ailments as a supplemental and alternative medicine in multiple illnesses, either raw or separated bioactive ingredients. The present study evaluated *in vitro* antioxidant and antimicrobial activities of the methanolic extract of the roots and flowers parts of *Asparagus racemosus* (*A. racemosus*) and *Clitoria ternatea* (*C. ternatea*), respectively. Total antioxidant capacity (T.A.C.) was determined using the phosphomolybdenum method. In vitro antimicrobial activity was assessed using the agar well diffusion method against four strains: E.coli, *Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. The methanolic crude extracts of *A. racemosus* and *C. ternatea* are subjected to thin-layer chromatography. The separation of spots was observed under daylight, shorter wavelength 254nm and longer wavelength 366 nm using different solvent systems. The Rf values were determined.The antioxidant activity of the methanolic extracts of *A. racemosus* and *C. ternatea* was remarkable and dose-dependent. Methanolic extracts of *A. racemosus* and *C. ternatea* were tested by agar well diffusion method against four selected strains to determine antimicrobial activity. All of the tested strains showed significant inhibitory action, indicating that *A. racemosus* and *C. ternatea* are promising sources of herbal medicine. These results suggest that *A. racemosus* and *C. ternatea* effectively scavenge free radicals and can be powerful antioxidants. As a result of the findings of this investigation, *A. racemosus* and *C. ternatea* extract could be considered a possible source of natural antioxidants. It has the potential to be used as an antibacterial agent.

Key words: Cold maceration,Thin layer chromatography,Total antioxidant capacity, Agar well diffusion method

INTRODUCTION

Infections induced by harmful microorganisms are becoming a significant source of mortality and morbidity in immunocompromised people in developed countries (Al-Bari *et al*.2006). Antimicrobial resistance must be controlled urgently by reducing hospital cross-infection andimproving antibiotic use (French, 2005), yet, novel antibiotics must be developed, as they are critical to maintaining antimicrobial therapy effectiveness (Vander waaji and Nord, 2000).

The WHO estimates that roughly 3/4th of the population in underdeveloped countries rely on plant-based remedies for their traditional medicinal system and human primary health care (W.H.O.2002). As a result, several medicinal plants have been tested for antimicrobial activity and to find cures for various microbial-related diseases,neurodegenerative disorders, atherosclerosis, cardiovascular dysfunction,carcinogenesis, inflammation, reperfusion injuryand medication toxicity all ofwhichinclude oxidative stress as a contributing factor in their pathogenesis (Rao, 2000; Subramani and Goraya, 2003). Plants (vegetables, fruits, and medicinal herbs) contain many free radical scavenging molecules, including nitrogen compounds,phenolic compounds,terpenoids, vitamins, and other endogenous metabolites with high antioxidant activity (Zheng and Wang, 2001; Cai *et al*.2003). Antioxidants that scavenge free radicals may aid

*Correspondence:reetatripathi10@rediffmail.com

in the slowed progression of fibrosis in the body.Antioxidants are potential protective agents in the human bodythat minimize oxidative damage (Yam *et al*.2008).Antioxidants are naturally abundant in fruits and can neutralize free radicals donating an electron and converting them to harmless molecules (Leonard *et al*. 2002). Natural antioxidants have a wide range of biochemical activities,including inhibition of R.O.S. generation, direct or indirect scavenging of free radicals, and intracellular redox potential (Abdolahi *et al*. 2005). An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in preventing degenerative diseases. In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant-rich food and the incidence of human diseases. *A. racemosus* (Shatavari, Asparagaceae) is a widely occurring medicinal plant found abundantly in tropical zones and subtropicallike India, Asia, Australia and Africa. The phytochemical constituents of the plant vary depending on its geographical zone of availability. *A. racemosus* is frequently used in Ayurvedic drug preparations as it is known to treat conditions such asboost immunity, aging, vigor, and improve longevity and mental function. *A. racemosus* also finds its application in curingdyspepsia, hepatopathy, neurological disorders, and tumors. Various therapeutic property of the root of *A. racemosus*is well documented in ancient Ayurvedic literature. The medicinal property is due tomultiple pharmacological properties such asanti-inflammatory, antioxidant, antimicrobial and antiseptic, properties. The major phytochemical constituents present in the roots of *A. racemosus* are steroidal saponins (Kamat *et al*. 2000; Acharya *et al*. 2012). *C. ternatea* (Fabaceae), known as Butterfly pea, is a perennial twining herb found in tropical equatorial areas. The plants are much adaptive to various ranges of humidity and temperatures. *C. ternatea* varieties contain phytochemical constituents with important pharmacological activities. Many secondary metabolites, including flavanol, triterpenoids, glycosides, steroids,and anthocyanins, are isolated from *C. ternatea*. Its extracts possess a wide range of pharmacological activities, including antipyretic,antimicrobial, analgesic,anti-inflammatory, local anesthetic, diuretic, antidiabetic, blood platelets aggregation inhibiting and insecticidal properties.This plant has an extended use in traditional Ayurvedic medicine for several diseases and scientific studies have reconfirmed with modern relevance(Rajesh *et al*. 2017). The present study determined the antioxidant activities of methanol extracts of *A. racemosus* and *C. ternatea*. Also, it was interesting to determine the antimicrobial activities of these extracts, which were carried out by the disc diffusion method.

MATERIALS AND METHODS

Plant materials

The roots and flowers of *A. racemosus* and *C. ternatea* were collected from the Vindhya herbal nursery of forest department Bhopal (M.P.) in January 2020. Plant material (roots and flowers) selected for the study were washed thoroughly under running tap water and then rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was ground using an electronic grinder. Powdered plant material was observed for itsodour,colour, texture andtaste. Dried plant material was packed in an airtight container and stored for phytochemical analysis.

Chemical reagents

All the chemicals used in this study were obtained from Sigma-Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), Hi-Media Laboratories Pvt. Ltd. (Mumbai, India),S.R.L. Pvt. Ltd. (Mumbai, India) and SD Fine-Chem. Ltd. (Mumbai, India). All the chemicals and solvents used in this study were of analytical grade. The pathogenic microbes used in the current study were obtained from the Microbial Culture Collection, National Centre For Cell Science, Pune, Maharashtra, India.

Preparation of solvent extract by cold maceration

Plant material was extracted by using the cold maceration method. Almost 500 g of the powder was successively extracted with different organic solvents, petroleum ether, ethyl acetate and methanol and allowed standing for 5 days each. The extract was filtered using Whatman No. 1 filter paper to remove all unextractable matter, including cellular materials and other insoluble constituents in the extraction solvent. The extract was

transferred to a beaker and evaporated; excessive moisture was removed and the extract was collected in an airtight container free from any contamination until it was used. Finally, the percentage yields were calculated for the dried extracts.

Thin-layer chromatography profiling of extracts

Thin-layer chromatography was performed to analyze the variation in bioactive chemical constituents. The T.L.C. plates were prepared using Silica gel 'G' as 30 g of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for 2 minutes; this suspension was distributed over the plate was air-dried until the transparency of the layer disappeared. The plates were dried in a hot air oven at 110°C for half an hour and then stored in a dry atmosphere and used whenever required. Samples were prepared by diluting the crude extracts with respective solvent and then applying usually 1-10ìl volumes to the origins of a T.L.C. plate, 02 cm above its bottom, with the help of capillary tubes. The mobile phase solvent systems used were chloroform: methanol (7:3) solvent system for *A. racemosus* and toluene: ethyl acetate: formic acid (7.8:1.8:0.4) solvent system for *C. ternatea* for the detection of phytoconstituents. The developed chromatograms were analyzed for the presence of drug constituents by spraying with an appropriate group reagent. The chromatograms were then observed under UV-254 nm and UV-365 nm light. Photos were taken with a Nikon camera and the R*f* values were calculated with the following formula (Mehta *et al*. 2017).

Rf = Distance travelled by solute/Distance travelled by the solvent

Antioxidant activity

Total antioxidant capacity (T.A.C.)

Determination of total antioxidant capacity was evaluated by the Phosphomolybdenum method (Prieto *et al*. 1999]. 0.3 ml of extract and sub-fraction in ethanol, ascorbic acid used as standard (5 to 200g/ml) and blank (ethanol) were combined with 4 ml of reagent mixture separately and incubated at 90°C for one and half hours. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid or Gallic acid. The antioxidant activity is described as the number of equivalents of ascorbic acid and was calculated by the following equation:

$$A=(C\times V)/m$$

where, V = the volume of extract (ml),
m = the weight of crude plant extract (g),
A = total content of antioxidant compounds, mg/g plant extract, in ascorbic acid equivalent,
C = the concentration of ascorbic acid established from the calibration curve mg/ml.

Antimicrobial activity

Method of preparation

This agar medium was dissolved in distilled water and boiled in a conical flask of sufficient capacity. Dry ingredients are transferred to a flask containing the required quantity of distilled water and heat to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in an autoclave for sterilization at 15 lbf /in^2 (121^{o}C) for fifteen minutes.

Preparation of plates

After sterilization, the media in the flask was immediately poured (20 ml/ plate) into sterile Petri dishes on a plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use.

Well diffusion method

The suitable diffusion method was used to determine the antimicrobial activity of the extract prepared from roots and flowers of *A. racemosus* and *C. ternatea* using standard procedure (Bauer *et al*. 1966).There were four concentrations used, which are 25, 50, 75 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. Its essential feature is placing the antibiotics on agar surfaces immediately after inoculation with the

organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37°C for one day and then examined for clear zones of inhibition around the wells impregnated with a particular drug concentration.

RESULTS AND DISCUSSION

Thin layer chromatography

Thin layer chromatographic analysis provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs and for detecting contaminants and substitutions. With the aid of appropriate separation procedures, T.L.C. can analyze drug combinations and phytochemical preparations. In the thin-layer chromatographic analytical method, many solvent systems were tried to achieve a good resolution. Finally, chloroform: methanol (7:3) solvent system for *A. racemosus* and toluene: ethyl acetate: formic acid (7.8:1.8:0.4) solvent system for *C. ternatea*. It was shown to have a good resolution in alcoholic extracts. Visibility of spots in long-wavelength, shorter wavelength, and visible light was examined separately. The number of spots, the colour of the spots and calculated *Rf* values are tabulated in Table 1 and corresponding figures of thin layer chromatography are in Fig. 1.

Table 1: Thin layer chromatography of methanolic extracts of plants

Plant Sample Extracts	Rf value	
A.racemosus	0.8	1
C.ternatea	0.5	0.7

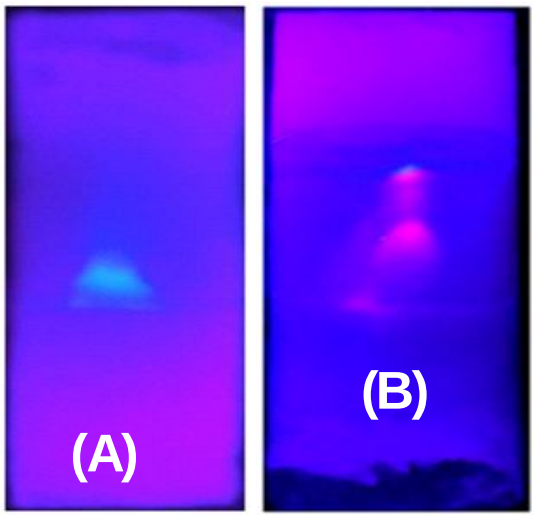

Fig. 1 : (A) T.L.C. image of *A. racemosus* (B) *C.ternatea*

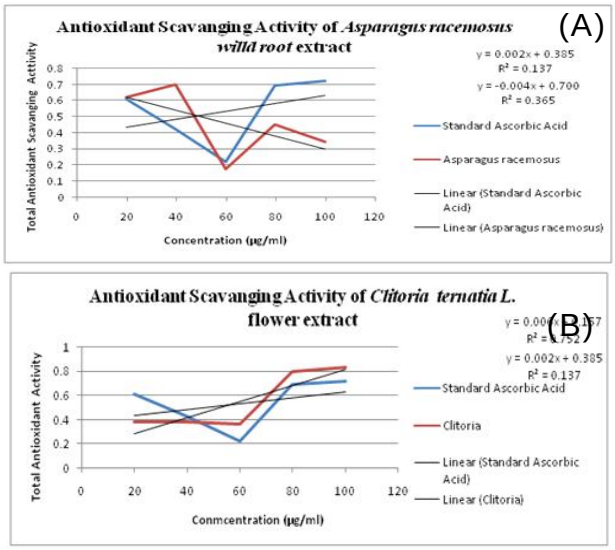

Fig. 2 : Total antioxidant activity (phosphomolybdate assay) of extract **(A)** *A.racemosus* **(B)** *C. ternatea*

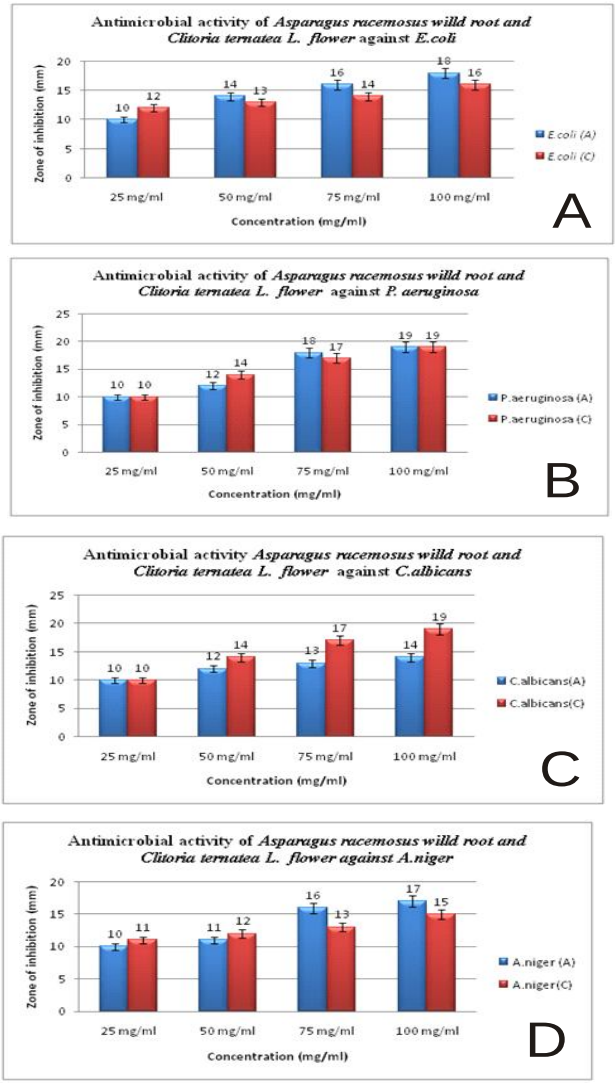

Fig. 3 : Antimicrobial activities of methanolic extracts of *A. racemosus* and *C. ternatea* against (A) *E. coli,* (B) *P. aeruginosa,* (C) *C. albicans*, (D) *A. niger*

Antioxidant activity

The total antioxidant capacity equivalent of ascorbic acid was 0.609 mg/g of extract. Concentrations ranging from 20-100 µg/ml of the extract of *A. racemosus* and *C. ternatea* were tested for their total antioxidant activity. The maximum peak value of antioxidant scavenging activity 0.699 was shown at 40 µg/ml compared with standard ascorbic acid equivalent. The minimum peak value of 0.177 was found at 60 µg /ml for *A. racemosus*. The maximum peak value of antioxidant scavenging activity 0.792 showed at 80 µg/ml compared with standard ascorbic acid equivalent and minimum peak value 0.367 were found at 60 µg /ml for *C. ternatea* (Fig.2).

Antimicrobial activities

The antimicrobial activity of methanolic extract of plantsshowed bioactivity by inhibiting the growth of microbial species selected for the test, as shown in Fig.3. The zone of inhibition exhibited by the extracts was comparable to the standard drug. It is effective against all microbes in a concentration-dependent manner.

CONCLUSION

Based on these results, it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms. They can be used to treat infectious diseases caused by resistant microorganisms. They can also be a source of natural antioxidants. Due to their antibacterial and antioxidant activities, *A. racemosus* and *C. ternatea* extracts have promising potential as a source of natural antioxidant and antimicrobial agents. Such screening of various natural organic compounds and identifying active agents is the need of the hour because the successful prediction of a lead molecule and drug-like properties at the onset of drug discovery will pay off later in drug development.

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