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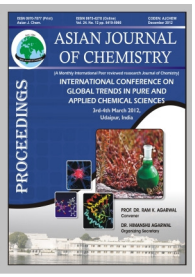

In vitro Screening of Combined Extracts of Some Medicinal Plants of Indian Origin for Antioxidant and Antibacterial Activity†

HUMA ALI* and SAVITA DIXIT

Department of Chemistry, Maulana Azad National Institute of Technology, Bhopal-462 051, India

*Corresponding author: E-mail: sana_soni26@yahoo.co.in

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The use of natural products as medicine has been practiced from ancient time in world. With an estimation of WHO that as many as 80 % of world's population rely on herbal traditional medicines as their primary health care. Based on local use of common diseases and ethnobotanical knowledge, an attempt has been made to investigate the antioxidant and antibacterial activity of combined extracts of *Aloe barbandesis* (leaves), *Tinospora cordifolia* (stem), *Azadirachta indica* (leaves) and *Ocimum sactum* (leaves). In this study the combine extract of selected plant materials, were analyzed for their antioxidant activity by peroxide value method and diphenylpicrylhydrazyl radical scavenging method along with the reference sample ascorbic acid. The extract were further analyzed to determine their total phenolic content by Folin-Ciocalteau method and total flavonoid content by Dowd method along with the reference sample rutin and gallic acid respectively. The extract exhibited significant antioxidant activity, total phenolic and flavonoid content. The combined extract also showed significant antibacterial activity against *Escherichia coli, Proteus, Staphylococcus aureus, Staphylococcus cohni*, *Klebsialla pneumonia*. The antibacterial activity was evaluated according to well diffusion method by using gram positive *S. aureus, S. cohni* and gram negative *E. coli, Klebsialla pneumonia, Proteus*. The result indicated that the combine extract had maximum antibacterial activity against, *Staphylococcus cohni* compare to the other microorganism. The extract can be used effectively for medication purposes.

Key Words: Medicinal plants, Antioxidant, Antibacterial.

INTRODUCTION

Natural products play an important role in drug development programs in the pharmaceutical industry[1]. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. In continuation of this strategy of new drug discovery we have studied the combined effect of *Azadirachta indica, Ocimum sactum, Aloe barbandesis and Tinospora cordifolia* for their antioxidant and antibacterial activities.

Tinospora cordifolia (part used stem) belongs to the family *Menispermaceae* is used as an ingredient for ayurvedic preparation in general debility, dyspepsia, fevers and urinary diseases[2]. *Aloe barbadensis* belongs to the *Liliaceal* family. *Aloe vera* contains over 75 nutrients and 200 active compounds, including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid and amino acids[3]. Numerous scientific studies on *Aloe vera* are demonstrating its analgesic, antiinflammatory, wound healing, immune modulating and antitumor activities as well as antiviral[4]. *Azadirachta indica* belongs to family meliaceae distributed widespread in the world. The chemical constituents contain many biologically active compounds that can be extracted from *Azadirachta indica*, including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Neem leaf is effective in treating eczema, ringworm, *etc*., *Ocimum sactum* belongs to the family Labiateae. The leaves yield an essential oil containing eugenol, carvacrol, methyl eugenol and possess various medicinal properties[5].

EXPERIMENTAL

Collection of plant material and preparation of extracts: Fresh leaves of *Azadirachta indica, Ocimum sactum, Aloe barbandesis,* stem of *Tinospora cordifolia* were collected from their proper origin. The separate plant materials were air-dried at room temperature (26 ºC) for 2 weeks, after which it was grinded to a uniform powder. The mixture of acetone and methanol (30:70) extract were prepared by using 75 g each of the dry powdered plant materials in soxhelet apparatus at 40 ºC for 48 h. The extract were filtered after 48 h, the extracts were concentrated using a rotary evaporator with the water bath at 40 ºC. The percentage yield of extracts ranged from 7-17 %

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w/w. The extract of each plant material were mixed in equal proportion and also make a combine sample of different extract.

Determination of antioxidant activity: Antioxidant activity of combined extract of above mentioned plants was determined against hydrogen peroxide and 2,2-diphenyl-1-picrylhydrazyl (DPHH) radical scavenging along with the reference sample ascorbic acid. The extract were further analyzed to determine their total phenolic content by Folin-Ciocalteau method and total flavonoid content by Dowd method along with the reference sample gallic acid and rutin respectively.

Determination of the total flavonoid content: For total flavonoid content different concentration of plant extract (1 mg/mL) and different concentration of rutin as a standard were taken. 0.5 mL of sample solution was taken and 2.5 mL of distilled water and 0.150 mL of 5 % $NaNO_2$ was added. After 6 min, 75 µL of 10 % $AlCl_3$ was added. After another 5 min, 0.5 mL of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm *versus* prepared water blank[6].

Determination of the total phenolic content: The amount of total phenolic in extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01 to 0.1, mg/mL of gallic acid were prepared in methanol. Concentration 1 mg/mL of plant extract were also prepared in methanol and 0.5 mL of each sample were introduced in to test and mixed with 2.5 mL of a 10 fold dilute Folin-Ciocalteu reagent and 2 mL of 7.5 % sodium carbonate. The tubes were covered with parafilm and allowed to stand for 0.5 h at room temperature before the absorbance was at read at 760 nm spectrometrically. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, there by producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined[7].

DPPH scavenging activity: 1 mg of extract powder were dissolved in 1 mL of 90 % methanol solution to obtain 1000 µg/mL sample solutions were series diluted in to concentration ranging from 400-1000 µg/mL (*i.e.* 400, 500, 600, 700, 800, 900 and 1000 µg/mL). 200 µM solution of DPPH in methanol was prepared and 1.5 mL of this solution was added to 1.5 mL of methanol extract solution at different concentrations (400-1000 µg/mL). Mixture of methanol and DPPH were used as the standard control. After 0.5 h, the absorbance was measured at 517 nm. The absorbance of DPPH solution decreases when kept in contact with antioxidant test sample and free radical scavenging activity is inversely proportional to the absorbance of DPPH solution[8,9]. Percent inhibition of DPPH free radical scavenging activity was calculated using the following formula. Ascorbic acid was used as a standard control. IC_{50} values denote the concentration of sample, which is required to scavenge 50 % of DPPH free radicals.

DPPH scavenged (%) = $(A_{cont} - A_{test}) / A_{cont} \times 100$, where A_{cont} is the absorbance of the control reaction. A_{test} is the absorbance in the presence of the sample of the extract.

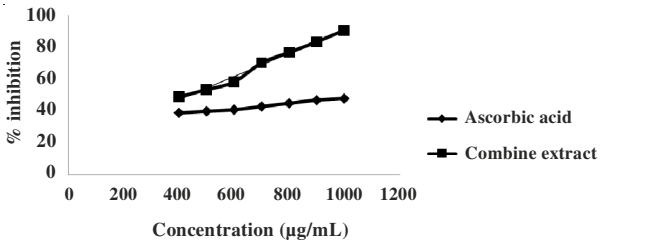

Fig. 1. Determination of IC_{50} values for standard and combine extract from linear correlation between concentrations (µg/mL) verses percentage of scavenging of DPPH

Scavenging of hydrogen peroxide: Sample with different concentration (*i.e.* 400, 500, 600, 700, 800, 900 and 1000 µg/mL) were added to 0.1 M phosphate buffer solution (pH 7.4, 3.4 mL) respectively and mixed with 43 mM H_2O_2 solution (0.6 mL). After 10 min, the reaction mixture absorbance was determined at 230 nm. The reaction mixture without sample was used as the blank[10]. Ascorbic acid was used as a standard control.

The % inhibition activity = $(A_{cont} - A_{test}) / A_{cont} \times 100$, where, A_{cont} is the absorbance of the control reaction. A_{test} is the absorbance in the presence of the sample of the extract.

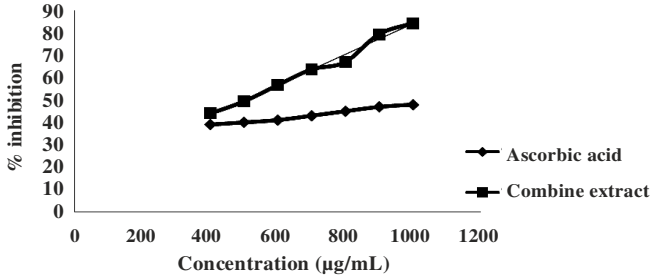

Fig. 2. Determination of IC_{50} values for standard and combine extract from linear correlation between concentrations (µg/mL) *verses* percentage of scavenging of H_2O_2

TABLE-1
TOTAL PHENOL AND FLAVONOIDS CONTENTS, DPPH AND H_2O_2 SCAVENGING ACTIVITIES OF COMBINE EXTRACT OF *Azadirachta indica, Ocimum sactum, Aloe barbandesis, Tinospora cordifolia*

Sample	Total phenol content (mg/g)	Flavanoid content (mg/g)	DPPH radical scavenging, IC_{50} (µg/mL)	H_2O_2 radical scavenging, IC_{50} (µg/mL)
Combine extract	13.75	16.14	514.1	504.7
IC_{50} for ascorbic acid was 498.1 µg/mL				

Determination of antibacterial activity: 7.6 g of Muller Hinton Media (Hi-Media) was mixed with 400 mL distilled water (pH 7.5) and then sterilized in autoclave at 15 lb pressure for 15 min. The sterilized media were poured in to Petri dishes. The solidified plates were bored with 5 mm diameter cork bearer. The plates with wells were used for the antibacterial studies. 1 g of the combine extract was dissolved in DMSO and of 400, 200, 100 and 50 mg concentration was tested against gram positive *Staphylococcus aureus, Staphylococcus cohni* and gram negative *Escherichia coli, Proteus* and *Klebsialla pneumonia* for their antimicrobial activity. It was demonstrated by well diffusion method.

TABLE-2
ANTIBACTERIAL ACTIVITY

S. No.	Concentration (mg)	Zone of inhibition (mm in diameter)				
		Escherichia coli	*Proteus*	*Staphylococcus aureus*	*Staphylococcus cohni*	*Klebsialla pneumonia*
1.	400	18.49	17.54	19.08	22.96	17.63
2.	200	16.27	17.71	17.98	18.58	17.15
3.	100	16.73	17.85	17.54	18.67	17.09
4.	50	15.23	16.21	17.34	17.94	15.28

Antibacterial activity of the combined extracts was tested using Well diffusion method[5]. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the media surface with 6 mm cork borer. The combine were poured in to the well using sterile syringe. The plates were incubated at 37 ºC for 24 h for bacterial activity. Each concentration of the combine sample was tested against different bacterial pathogens. It was demonstrated by well diffusion assay. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (mm) including the well diameter. The readings were calculated in four different fixed directions and average values were calculated.

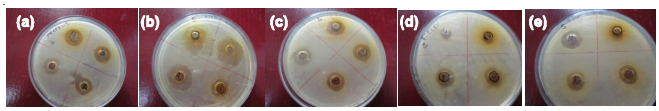

Fig. 3. Antibacterial activity of combine extract of *Aloe barbandesis, Tinospora cordifolia, Azadirachta indica* and *Ocimum sactum* against *Proteus* (A), *Klebsialla pneumonia* (B), *Staphylococcus cohni* (C), *Escherichia coli* (D) and *Staphylococcus aureus* (E)

RESULTS AND DISCUSSION

The percentage yield of *Azadirachta indica, Ocimum sactum, Aloe barbandesis, Tinospora cordifolia* was found to be 7-17 % w/w and total phenol compounds, as determined by Folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y = 0.0032x + 0.009$, $R^2 = 0.984$). The total flavonoid contents are reported as mg rutin equivalent/g of extract powder, by reference to standard curve ($y = 0.0007x + 0.049$, $R^2 = 0.989$) (Table-2). It was noted that this combined extracts possess higher total phenol and flavanoids. Phenols and polyphenolic compounds, such as flavonoids, are derived from plant sources; possess significant antioxidant activities[8].

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples[11]. DPPH is a stable nitrogen-centered free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers[12]. It was found that the radical-scavenging activity of extract increased with increasing concentration with IC_{50} 514.1 µg/ mL usually, higher total phenol and flavonoid contents lead to better DPPH-scavenging activity[13].

Scavenging of H_2O_2 by extract may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water[14]. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method where they are compared with that of gallic acid as standard. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner with IC_{50} 504.7 µg/mL. The antibacterial activity of the combine extract was analyzed against *Escherichia coli, Proteus, Staphylococcus aureus, Staphylococcus cohni, Klebsialla pneumonia*. The maximum antibacterial activity was observed against *Staphylococcus cohni* compare to the other micro organism.

Conclusion

The present study revealed that the combined extracts has profound antioxidant and antibacterial effect and may have potential use in medicine. The extract showed moderate to maximum antimicrobial activity against the tested microorganism. It may be concluded from this study that this extract may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radical and active against the tested microorganism.

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