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COMPARATIVE EVALUATION OF *IN VITRO* ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY USING STANDARD DRUG AND POLYHERBAL FORMULATION

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ABSTRACT

The present study was taken to investigate the *in vitro* antibacterial and antioxidant activity of polyherbal formulation comparative to standard drug and individual plant extract. Cup and Plate method was used for *in vitro* antibacterial screening. Zones of inhibition were observed in disc diffusion for antibacterial investigation against Gram-positive and Gram negative pathogenic bacteria. The Polyherbal formulation showed average zone of inhibition comparative to individual plant extract and standard drug ranged from 9-12 mm. In antioxidant screening, the compound showed significant.

Keywords: Polyherbal formulation, Cup and Plate method, Antibacterial and Antioxidant activity, FRAP assay.

INTRODUCTION

Medicinal plants are widely exploited worldwide for their active ingredients. This has created a wide gap between the production and exploitation of most of the medicinal plants of important plant species.

The Siddha System of Medicine (Traditional Tamil System of medicine), which has been prevalent in the ancient Tamil land, is the foremost of all other medical systems in the world. The uniqueness of Siddha System is evident by its continuous service to the humanity for more than 5000 years in combating diseases and also in maintaining its physical, mental and moral health while many of its contemporaries had become extinct long ago.Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Scientific experiments since the late 19th century have documented the antioxidant properties of some spices, herbs, and their components (Zaika, 1988; Bajpai *et al*., 2005). Many studies reported the activities of spices and herbs on food borne pathogenic microorganisms (Arora and Kaur, 1999; Yano *et al*., 2006). As such developing a polyherbal formulation with definitely produce synergistic effect as needed comparable to standard drugs that are available in mark*et al*l over the world. The purpose of the present study was to investigate the antioxidant and antimicrobial properties of *Withania somnifera*, *Bacopa monnieri and cinnamomum zeylanicum* indiviudaly and combination of all this three as polyherbal treatment. In this paper we report the results of such studies in order to orient future investigations towards the finding of new, potent and safe antioxidant and antimicrobial compounds.

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***Withania somnifera* is** a hedge plant that blooms in India and also the North America. The roots of the ashwagandha plant have been in use for treatment by Ayurvedic healers. It contains phyto-androgens, which are the precursors to reproductive hormones. Furthermore, this plant helps in reproductive health for men and women. The active constituents are slightly sedative in nature and it calms the central nervous system. It is reported that ashwagandha shows a great effect in reducing inflammation, increasing mental activity, invigorating the body, decreasing stress and as an antioxidant.

Bacopa monnieri is the small herb with the numerous branches. It grows to a height of 2 -3 feet and its branches are 10 -35 cm long. It has oval shaped leaves that are 1-2 cm long and 3- 8 mm broad. Leaves are formed in pairs along the stems. Small- tubular, five pedaled flowers are white- purple in colour. Its stem is soft, succulent, and hairy with the glands. Roots emerge out of the nodules and directly go to the soil. The fruit is oval and sharp at apex. It contains triterpene, saponins of the dammarane class, which are bacosides and bacopasaponins, and which contain 2 or 3 sugars each. Other constituents include mannitol, common plant sterols, and betulinic acid, as well as glutamic and aspartic acids. It is reported that brahmi shows a great effect in treating asthma, hoarseness, insanity, epilepsy, nerve tonic, cardio tonic and also diuretic.

Cinnamon common name is true cinnamon and its scientific name is *cinnamomum zeylanicum*.The ornamental is of a tree with golden red bark that is dried and is the cinnamon spice .It can grow well in soil which contains lot of sand and it grows at tropical climate.New foliage is deep red, and small white flowers are followed by dark purple fruit .the leaves contain eugenol and are sometimes used as a substitute for cloves and represent the major aromatic chemical component of cloves. Native region is from Sri Lanka, and Burma.The useful chemical component is cinnamon's bark is its oil which is just 4 % but others are cinnamaldehye(3-phenyl-acrolein,65 to75%) and eugenol (4-(1-propene-3-yl)-2-methoxy-phenol,5to 10%)which is also determine the flavor and aroma. its proven to have many pharmacological effects such as anti microbial,anti-inflammatory,antioxidant, and also used for type 2 diabetes.

MATERIALS AND METHODS

Preparation of Formulation

Readymade extracts of *Withania somnifera*, *Bacopa monnieri* and *cinnamomum zeylanicum* extract is obtained from department of ayurveda, Banarus Hindu University, Varanasi. Use to determine their antibacterial and antioxidant activity.

Comparison between individual plant extract, poly herbal formulation and standard drug has been done using cup and plate method. The standard pathogenic bacteria cultures were used in this study. The bacterial cultures were rejuvenated in Mueller- Hinton broth at 37 degree Celsius for 24hours. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37 degree Celsius for 3 hours.

Preparation of poly herbal formulation

Polyherbal formulation was prepared by simple percolation process. Distilled water was used to make up the volume up to 5ml.

All of the chemicals used in this work were obtained from Masterskill University College of Health Sciences Laboratory. The chemicals were analytical degree.

Biological materials

Three bacteria cultures were used in the study: Klebsiella aerogenes, Pseudomonas aeruginosa and Escherichia coli. The microorganisms maintained on Nutrient Agar and Muller Hinton (Merck, Darmstadt, Germany) were supplied by Microbiology Laboratory of Masterskill University College of Health Sciences.

Antibacterial Screening

Antibacterial Activity using cup and plate method

The cup and plate method method was employed to determine the antibacterialactivity of poly herbal preparations. Turbidity of inoculums was matched with McFarland turbidity standard. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Vancomycin antibiotic disc were used. The plates were incubated for 24 hours at 37 ^{0}C. The antibacterial activity was evaluated for 0.2 ml of each plant extract and polyherbal extract. The diameter of inhibition zones were measured and recorded.

In vitro antioxidant activity

Ferric Ion Reducing/antioxidant Power Assay (FRAP)

Antioxidant activity was determined by Ferric ion reducing antioxidant power assay (FRAP) as described by Oyaizu. 2ml of different plant extract (40mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 0.1 % potassium ferricyanide (2.5 ml). The mixture wasincubated at 50 ºC for 20 min. Aliquots of 10 % trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric

chloride solution (0.5 ml, 0.1%). The absorbance was measured at 640 by using UV Spectrophotometer.

Statistical evaluation

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of two replicates.

RESULTS AND DISCUSSION

Besides, the reducing ability of extract on antibacterial activity was determined, for the measurements of reducing ability, the transformation of Fe3+- Fe2+ in the presence of extract was adopted. The reducing power of the extract increased with the concentration. However, the absorbance remained constant after 400 mg/ml concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

Table 1. Composition of Polyherbal Formulation

Botanical name	Weight	Parts used
Withania somnifera	100mg	**whole plant**
Bacopa monnieri	100mg	**whole plant**
Cinnamomum zeylanicum	100mg	**whole plant**

Table 2. In vitro antibacterial activity of Individual plant extract, polyherbal formulation and standard drug

Test organism	**Diameter of zone of inhibition (in mm)**				
	BE	**WS**	**CZ**	**PH**	**VN**
Gram positive bacteria					
Klebsiella aerogenes	**16**	**17**	**17**	**19**	**23**
Gram negative bacteria					
Pseudomonas aeruginosa	**16**	**14**	**16**	**17**	**22**
Escherichia coli	**17**	**17**	**16**	**16**	**24**

Table 3. Antioxidant activity of polyherbal formulation

Concentration in µg/ml	Absorbance at 640 nm
100	0.63
200	0.68
300	0.93
400	1.07
500	1.10

Figure 1. Standard antibiotic disc before incubation

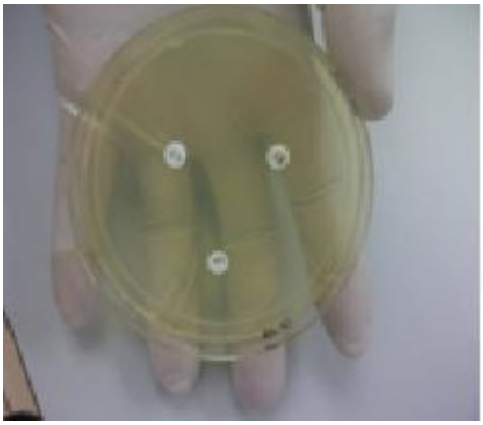


Figure 2. Zone of inhibition of standard antibiotics

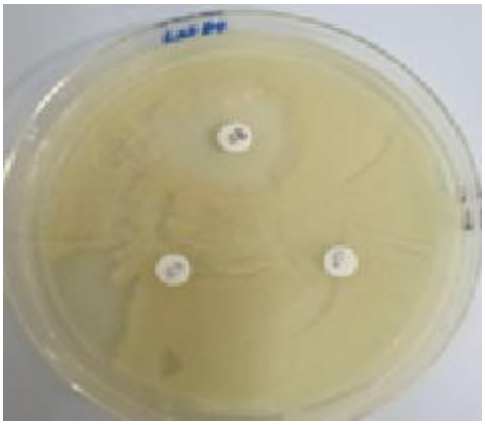


Figure 3. Zone of inhibition of Brahmi Extract

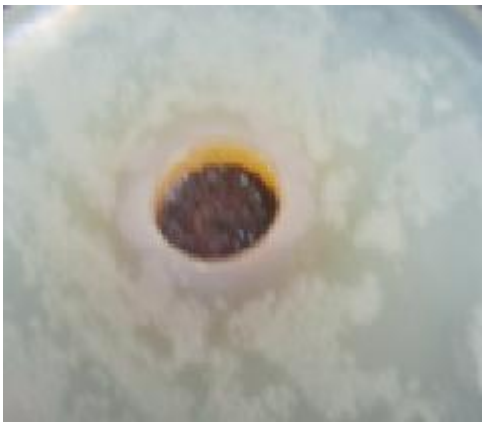


Figure 4. Zone of inhibition of Cinnamon Extract

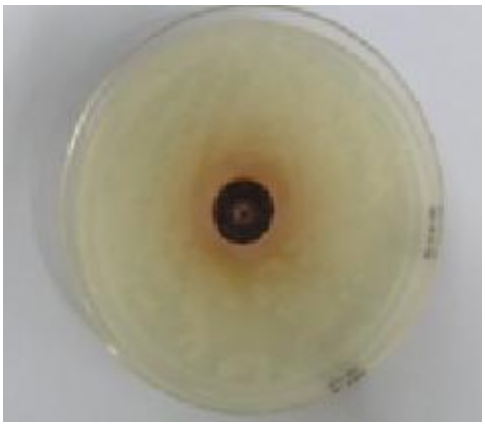


Figure 5. Zone of inhibition of Aswagandha Extract

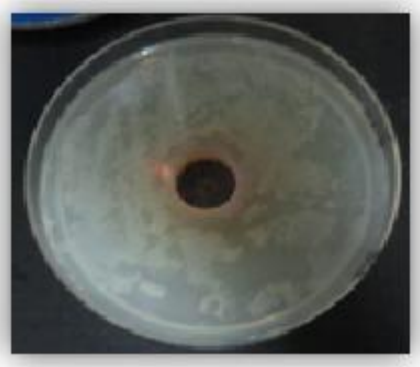
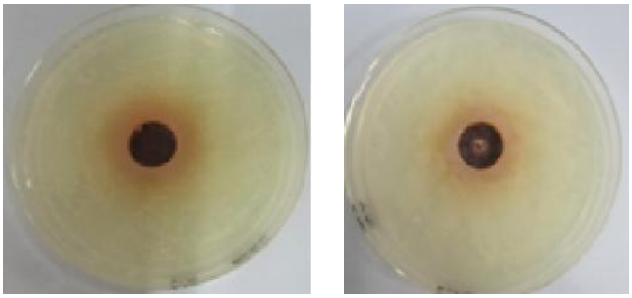


Figure 6. Greater inhibition zone of polyherbal formulation in Pseudomonas aeruginosa and Klebsiella aerogenes



CONCLUSION

The present study showed that Polyherbal formulation has got antibacterial and antioxidant activity. The Polyherbal formulation showed moderate to mild antibacterial activity against most of the tested bacteria. It may be concluded that Polyherbal formulation is active against the tested microorganisms and have good antioxidant activity. Further studies should be done to study on toxicity and clinical studies in order to develop a future drug containing this poly herbal formulation for treatment of infection and cancer.

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Declaration of interest

The authors report no conflicts of interest.

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