

Polyherbal Formulation as Potent Nutraceutical: Antioxidant and Antibacterial Activity

S J Kabilan, R Baskar, G Poorani

Abstract: The objectives of the study were to assess the potential of herbal formulation made up of *Wedeliachinensis* leaves and *Boerhaaviadiffusa* roots in possessing activities like antioxidant and antibacterial. The antioxidant potentials of the extracts were evaluated by performing DPPH radical scavenging assay and FRAP assay. The antibacterial activity was assessed using agar well diffusion method against choosed gram positive and gram negative bacterial strains. The results of the antibacterial and antioxidant activities showed that the formulation mixture of herbs *Wedeliachinensis* and *Boerhaaviadiffusa* roots is an excellent source of nutraceutical with high therapeutical importance.

Keywords: Antioxidant, Antibacterial, *Boerhaaviadiffusa*, *Wedeliachinensis*

I. INTRODUCTION

Wedeliachinensis (Manjalkarisalai in Tamil), Asteraceae is a well reported herbal medicine in Siddha, Unani and Ayurvedha system of traditional medicine. Recent studies show the presence of diterpenes, flavanoids, triterpenes, phytosteroids and saponins. It is also reported to possess anti-inflammatory, antioxidant, analgesic, hepatoprotective, antimicrobial, CNS depressant, wound healing, antistress and anticancer activity [6].

Boerhaaviadiffusa (Mookirattaikeerai in Tamil) is one of the well known medicinal plants that are used to treat variety of human diseased conditions as mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita [13]. Huge variety of phytochemicals like flavonoids, alkaloids, glycosides, rotenoids, steroids, lipids, triterpenoids, lignans, carbohydrates, glycoproteins and proteins etc have been reported from the herb [12]. The promising therapeutic effects of this plant include diuretic, hepatoprotective, anti-inflammatory, anti-cancer, anti-fibrinolytic, immuno-modulatory, anti-diabetic, immuno-suppressive, analgesic, anti-lymphoproliferative and used for the treatment of TB [14].

The aim of this study is to assess the polyherbal formulations ability to market as a potent Nutraceutical and also to assess the antioxidant and antibacterial efficiency.

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II. MATERIALS AND METHODS

A. Chemicals

DPPH, methanol, ethanol, ferric chloride, TPTZ, sodium carbonate, Nutrient broth, Nutrient Agar.

B. Sample collection and extraction

Wedeliachinensis leaves and *Boerhaaviadiffusa* root powder were collected from an FSSAI approved herbal powder manufacturer from Coimbatore, Tamil Nadu. All herbs were stored in sealed container for future use. The samples were labeled as the *Wedeliachinensis* leaves (WC), *Boerhaaviadiffusa* (BD) and Formulation mix (F MIX).

Sample extraction

About 20g of powdered F MIX of the polyherbal formulation was successively extracted using Soxhlet extraction method with 150 ml of distilled water. Then it is allowed to evaporate in open air to obtain aqueous extracts.

C. In Vitro Antioxidant Activity

DPPH Radical Scavenging assay (Alam et al. 2013)

The DPPH radical scavenging assay is based on the reduction of DPPH by the scavenging activity of antioxidants present in the extracts by the donation of protons. This was indicated by the change of color from purple to yellow which was measured by decrease in absorbance at 517 nm. 0.5ml of DPPH reagent was added to 2ml of test samples prepared in different concentrations (100 µg/ml - 600 µg/ml) using methanol. Methanol with DPPH used as control and methanol alone as blank. The percentage inhibition or reduction of DPPH with reference to control is analyzed as the final result. The IC50 value was also determined.

Formula for finding out the percentage inhibition:

$$[(\text{OD of control} - \text{OD of test}) / \text{OD of control}] * 100$$

where OD indicates the optical density or absorbance. A graph was plotted for the inhibition percentage vs concentration from which the value of IC50 is obtained.

Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain 1996)

In this assay the reduction of ferric tripyridyltriazine to ferrous tripyridyltriazine is indicated by the appearance of intense blue color which be monitored at 593 nm. 90µl of test sample (20 µg/ml - 300 µg/ml) was added to 2.7ml of the FRAP reagent and 270 µl of water was added finally after which the reaction mixture was incubated at 37oc for 30 minutes. The increase in absorbance of the mixture was noted at 593 nm.

D. Determination of Antibacterial activity

The antibacterial activity of the aqueous extract of formulation mix was assessed by agar well diffusion method against two Gram positive bacteria and three Gram negative bacterial test pathogens. Extracts were reconstituted to a final concentration of 10 mg/ml. DMSO was used as control in one of the wells. Each bacterial strain was inoculated in the Nutrient agar and from this 100 µl is taken and spread on each plate. Then 100 µl of the extracts were taken and added into the wells which were created by making punches of 6 mm diameter. These plates were then kept for incubation for 24 hrs at 37°C. The diameter of the zone of inhibition was measured in millimeters to assess the antibacterial activity of the formulation extract.

III. RESULTS AND DISCUSSION

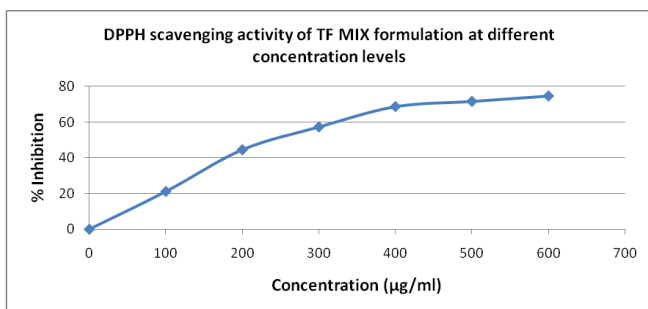
A. In vitro Antioxidant activity

DPPH Radical Scavenging assay (Alam et al. 2013)
 The change in color from violet to pale yellow indicates that free radicals have been scavenged by the extracts. This property allows visual monitoring at 517 nm. The scavenging activity in terms of % inhibition of F MIX extract is given in Table I. The production of oxidative stress by free radicals causes cellular damage. Oxidative stresses have been related to cancer, inflammation, ischemic injury and neurodegenerative diseases.

Table I: Scavenging effect (in percentage) on DPPH radical by F MIX formulation

| Sample concentration (µg/ml) | 100 | 200 | 300 | 400 | 500 | 600 |
|------------------------------|--------------|-------------|-------------|-------------|-------------|-------------|
| % Inhibition | 21.14 ± 1.69 | 44.5 ± 1.97 | 57.2 ± 0.96 | 68.6 ± 0.86 | 71.5 ± 0.84 | 74.5 ± 0.56 |

Fig. 1: Scavenging effect (in percentage) on DPPH radical by F MIX formulation at different concentration levels



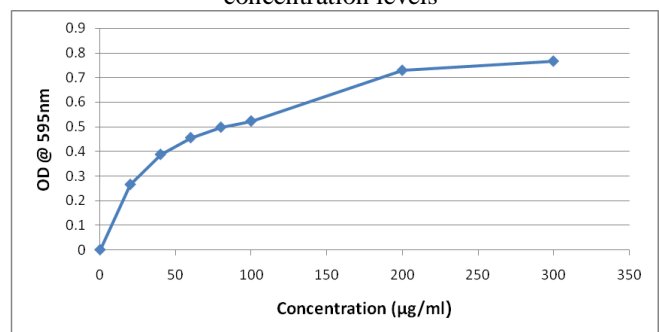
The IC50 value was found to be 243.72 µg/ml. This value proves that our F MIX formulation possesses better scavenging effect at lower concentrations. The antioxidant activity plays key role in the protection against toxicity -induced hepatopathy and nephropathy (Hassan et al., 2010)
 FRAP assay

The presence of antioxidants in the sample was indicated by the formation of intense blue color complex with increase in absorbance at 593nm. The antioxidant activity of different concentration of F MIX extract is given in Table II.

Table II: FRAP by F MIX formulation

| Sample Concentration (µg/ml) | Absorbance @ 595nm |
|------------------------------|--------------------|
| 20 | 0.266 ± 0.005 |
| 40 | 0.388 ± 0.006 |
| 60 | 0.456 ± 0.006 |
| 80 | 0.499 ± 0.008 |
| 100 | 0.524 ± 0.006 |
| 200 | 0.73 ± 0.009 |
| 300 | 0.767 ± 0.003 |

Fig. 2: FRAP by F MIX formulation at different concentration levels



The various radical scavenging assays revealed that extract can effectively prevent the biomolecules such as DNA, polyunsaturated fatty acids, amino acids, proteins from the damage caused by the attack of reactive radical species (Kanniparambil et al., 2017).

B. Determination of antibacterial activity

Two gram positive (*Enterococcus faecalis*, *Staphylococcus aureus*) and three gram negative (*E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) bacterial strains were used to evaluate the antibacterial activity of the polyherbal extract. Inhibitory concentrations used for the extract were 5mg/ml and of crude extract. Antibacterial activity was analyzed against the standard streptomycin antibiotic disc. The zone of inhibition of the F MIX extract against various bacterial species is tabulated in the Table III.

Table III: Antibacterial activity of polyherbal formulation extracts against various bacterial strains using the agar well diffusion method

| Organism | Diameter - Zone of Inhibition (in mm) | | | | |
|--------------------------|---------------------------------------|------------------------------|-------------------------|------------------------------|------------------------------|
| | <i>Pseudomonas aeruginosa</i> | <i>Klebsiella pneumoniae</i> | <i>Escherichia coli</i> | <i>Staphylococcus aureus</i> | <i>Enterococcus faecalis</i> |
| Crude extract | 18 | 16 | - | 14 | 11 |
| Diluted (5mg/ml) extract | 20 | 18 | - | 16 | 13 |
| Std. Streptomycin | 25 | 20 | 23 | 19 | 16 |

From the above table, it is evident that the F MIX formulation shows significant antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Enterococcus faecalis*. But, it did not show any appreciable effect over *E.coli*. These observations show that the formulation can prevent the infections against these organisms effectively. Prevention from bacterial infection itself will help the organ failure up to certain extent (Mistilis et al., 1970).

IV. CONCLUSION

The results of the antibacterial and antioxidant activities showed that the formulation mixture of herbs *Wedeliachinensis* and *Boerhaaviadiffusa* roots is an excellent source of Nutraceutical with high biomedical importance. Antioxidant property proves that it can also act as anticancer agent and in anti-aging ailments. The antibacterial activity of the mixture shows that it can be used as preventive medicine against many bacterial infections.

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Recent Publications:

- **Baskar, R.**, Akshaya, S.B., Akshitha, R., Dhilip Kumar, G., Poorani, G. (2018). Evaluation of antioxidant and phytochemical activity in solvent extracts of *Delonix regia*. *International Journal of Green Pharmacy*, Volume 12(3), July-September issue, S607-S616.
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- Gurumallesh, P., Alagu, K., **Ramakrishnan, B.** and Muthusamy, S (2019). A systematic reconsideration on proteases. *International Journal of Biological Macromolecules*, 128, 254-267 (**Impact Factor: 4.7**)
- Poorani Gurumallesh, **Baskar Ramakrishnan** and Bhaarithi Dhurai (2019). A novel metalloprotease from banana peel and its biochemical characterization. *International Journal of Biological Macromolecules*, 134, 527- 535 (**Impact factor: 4.7**)



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Notable Publications:

INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES (2019) IF 4.78 A novel metalloprotease from banana peel and its biochemical characterization

MATERIAL SCIENCE AND ENGINEERING C (2019) IF 5.08 Biological synergy of greener gold nanoparticles by using *Coleus aromaticus* leaf extract

INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES (2019) IF 4.78 A systematic reconsideration on proteases

PROCESS BIOCHEMISTRY (2019) IF 2.88 Green synthesis of anisotropic silver nanoparticles from the aqueous leaf extract of *Dodonaea viscosa* with their Antibacterial and Anticancer activities

JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B: BIOLOGY (2018) IF 4.06 Improved Conductivity and Antibacterial activity of poly (2- aminothiophenol) - silver nanocomposite against human pathogens

JOURNAL OF MICROENCAPSULATION (2016) IF 2.04 Formulation, characterization, in vitro and in vivo evaluation of castor oil based self-nano emulsifying levosulpiride delivery systems