



**COMPARATIVES ANTIOXIDANT POTENTIAL OF THREE PLANTS
BOERHAAVIA DIFFUSA, *KAEMPFERIA GALANGA* AND *BASELLA ALBA***

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Abstract

In the human body, free radicals and other reactive oxygen species are created throughout a variety of physiological and biochemical activities. An increase in these free radical generation can lead to oxidative damage to biomolecules (including lipids, proteins, and DNA), which can eventually result in many chronic diseases. Plethora of antioxidants found in plants serve to provide protection against diseases linked to free radicals. Thus this study aims at comparing antioxidant potential of three plants *Boerhaavia diffusa*, *K. galanga* & *Basella alba*. The plant material was collected & extracted with different solvents & further assayed for qualitative & quantitative study by standard procedures. Results revealed that Hydroalcoholic extract yielded maximum amount of phytochemicals. Also, the TPC & TFC was observed to be highest for *Basella alba* leaves extract. Further the IC₅₀ value for *Basella alba* was lowest it can be concluded that leaves of *Basella alba* have better antioxidant potential as compared to Roots of *Boerhaavia diffusa* and Rhizome of *Kaempferia galanga*.

Keywords: Medicinal plants, Phytochemicals, antioxidants, *Boerhaavia diffusa*, *Kaempferia galanga* and leaves of *Basella alba*, DPPH

Introduction

The human body goes through a natural and important process called oxidation. On the other hand, oxidative stress happens when there is an imbalance between the activity of free radicals and antioxidants. Oxygen-containing molecules with an unbalanced number of electrons are known as free radicals. Free radicals can interact with other molecules quite easily due to the unequal distribution of electrons. Free radicals can produce lengthy chemical chains in your body because they interact with other molecules so quickly. Free radicals can begin harming the fatty tissue, DNA, and proteins in your body when there are more of them than can be controlled by antioxidants. Your body is largely composed of proteins, lipids, and DNA, thus any damage can eventually result in a wide range of illnesses (Aruoma, 1998; Shinde *et al.*, 2012).

Antioxidant defences against free radicals are present in the body naturally. Antioxidants delay or stop

the oxidation of proteins, carbohydrates, lipids, and DNA, which can protect cells from free-radical damage and block the oxidation process even at low concentrations. Electrons can be donated by antioxidants. Strong electron donors, antioxidants stop the chain reaction by interacting with free radicals before important molecules are harmed. As a result, the antioxidants become oxidised and need to be replaced or renewed. Antioxidant enzymes, often found in cells, catalyse the destruction of several free radical species. The generation of extremely reactive hydroxyl radicals is prevented by transition metal binding proteins by preventing the interaction of transition metals, such as iron and copper, with hydrogen peroxide and superoxide (Kunwar&Priyadarsini, 2011; Kimet *al.*, 2004). Numerous investigations have been conducted to look for an effective and secure antioxidant based on the oxidative stress theory in various degenerative diseases and ageing. A small number of natural and synthetic antioxidants have been produced for therapeutic use despite promising in vitro investigations because of their low efficacy and undesirable side effects. Plethora of antioxidants found in plants serve to provide protection against diseases linked to free radicals. Plants create the majority of the antioxidant chemicals as secondary metabolites. Phytochemicals have a variety of positive health effects and disease-prevention capabilities. They include non-essential nutrients, meaning that the body does not need them to function and maintain life. Plants create these substances in order to maintain life, and when humans consume them, they benefit from the health benefits. Based on their function in plant metabolism, the over a thousand known phytochemicals can be divided into primary and secondary constituents (Kumar et al., 2008; Zahinet *al.*, 2009). Thus this study aims at comparing antioxidant potential of three plants *Boerhaavia diffusa*, *K. galanga* & *Basella alba*.

Boerhaavia diffusa is a herbaceous member of the family Nyctaginaceae. can be found all across the tropics and subtropics. Indigenous and tribal peoples have traditionally used it, and it is also used in Ayurvedic or natural herbal medicines. *B. diffusa* is used to treat a variety of ailments in Ayurvedic medicine in India and Unani medicine in Arab countries, including diabetes, stress, dyspepsia, gastrointestinal pain, inflammation, jaundice, spleen enlargement, and congestive heart failure. This plant is mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita as a remedy for a variety of human afflictions (Goyal et al., 2010; Maheshet *al.*, 2012).

K. galanga is from a dried rhizome of herb *Kaempferia galanga* L., belonging to the important family Zingiberaceae and genus *Kaempferia*. Due to its effective treatment of rheumatism, dry cough, colic, muscle discomfort, inflammations, and tumours, it was utilised as a folk medicine. In India, *K. galanga* was utilised for postpartum care for women as well as the treatment of intestinal wounds and urticarial. Additionally, it could be used as a food condiment. Phlegm, fever, and cough were all conditions that *K. galanga* was used to treat, and it also works well as a diuretic, anabolic, and carminative (Khairullahet *al.*, 2021 Kumar, 2020).

Basella alba belongs to the Basellaceae family. It is a significant ethno-vet medicinal plant that is also used to cure anaplasmosis, retained after birth, balanitis, and gonorrhoea. Chronic ; headaches can be treated with the mucilaginous liquid made from the plant's leaves and sensitive stalks. In addition to being a helpful laxative for youngsters and pregnant women, a decoction of the leaves was also observed to have a positive impact on men's total-body vitamin A reserves. It has a tremendous amount of potential to affect diabetes, and this has been proven scientifically (Swati & Agrawal, 2015; Chaurasiya et al., 2021).

Materials & Methods

Collection of plant material

Roots of *Boerhaavia diffusa*, rhizomes of *Kaempferia galanga* and leaves of *Basella alba* were collected from local area of Bhopal month of June, 2020.

Defatting & extraction

142 gram of dried powdered of *Boerhaavia diffusa*, 77 gram of dried powdered of *Kaempferia galanga* and 80 gram of dried powdered of *Basella alba* extraction was continued till the defatting of the material had taken place.

Defatted dried powdered has been extracted with successive solvents like chloroform, ethyl acetate, hydroalcoholic (80:20: ethanol: water) and water using maceration process for 24 hrs, filtered and dried using vacuum evaporator at 40°C.

Qualitative tests for phytochemicals

The screening for phytochemicals was performed as per standard test procedure.

Total phenol content

TPC of plant extracts was assessed using the Folin-Ciocalteu colorimetric technique. 2 mL of extract which was prepared by dissolving 10 mg of extract in 10ml of methanol distilled water was taken. In

that 1 mL of Folin-Ciocalteu reagent, and 1 mL of a of sodium carbonate was added. At room temperature, the mixture was incubated for 10 minutes. The absorbance was then assessed at a wavelength of 765 nm using a spectrophotometer. The gallic acid standard curve was used to calculate the total phenolic content. Gallic acid was dissolved in distilled water to create a stock gallic acid standard solution that contained 1 mg/ml. The result was represented as mg gallic acid equivalent/100mg (Sulaiman & Balachandran, 2012).

Total flavonoid content

The total flavonoid content of the extracts was assessed using the aluminium chloride complex-forming assay. The flavonoid concentration was determined to be the quercetin equivalent using quercetin as the benchmark. 10 mg quercetin was dissolved in 10ml methanol. 10 mg of dried extract was dissolved in

10 ml methanol. 3 ml of extract was then mixed with 1 ml of 2 % AlCl₃. Further incubation period of 15 min was set. In UV spectrophotometer, the absorbance of this reaction combination was noted at 420 nm. The amount of flavonoids was determined as mg QE/100mg of quercetin equivalents (Chang *et al.*, 2002).

Antioxidant activity by DPPH

By using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, which is a method previously published, the free radical scavenging activity of the fractions was assessed in vitro. 6 mg of DPPH was dissolved in 100 ml of methanol to create the stock solution, which was then kept at 20°C until needed. Using a spectrophotometer, DPPH solution was diluted with methanol to produce the working solution, which had an absorbance of around 0.980.02 at 517 nm. In order to test different concentrations (10–100 µg/ml), a 1.5 ml aliquot of this solution was combined with 1.5 ml of the sample. After thoroughly shaking, the reaction mixture was allowed to sit at room temperature for 15 minutes in the dark. Next, the absorbance at 517 nm was measured. As stated previously, the control was created without any sample. The amount of DPPH radicals that were successfully scavenged was used to determine the scavenging activity (Mensoret *et al.*, 2001).

Results & discussion

In case of *Boerhaavia diffusa* maximum phytochemicals are found to be present in hydroalcoholic extract. The phytochemical that gave positive results were for almost all phytochemicals except alkaloid & tannin. In case of *Kaempferia galanga* similar amount of phytochemicals were found to be present. In

the third plant *Basella alba* also the maximum phytochemicals were observed in hydroalcoholic extract. The ethyl acetate extract was found to be devoid of any phytochemicals for all the three plants.

For *Boerhaavia diffusa* the phenol & flavonoid content in hydroalcoholic extract was found to be 0.885 mg/100 mg & 0.842 mg/100 mg respectively. For aqueous extract the flavonoid content was found to be 0.523 mg/100 mg.

In case of *Kaempferia galanga* the observed phenol & flavonoid content in hydroalcoholic extract was recorded as 0.353 mg/100 mg & 0.756 mg/100 mg. While the aqueous extract of *Kaempferia galanga* noticed to have phenol & flavonoid content as 0.167 mg/100 mg & 0.396 mg/100 mg respectively.

For the third plant *Basella alba* the estimated phenol & flavonoid content was 0.842 mg/100 mg & 0.890 mg/100 mg. The aqueous extract of same plant estimated to have total flavonoid content as 0.457 mg/100 mg. Thus, from the obtained values of phenol & flavonoid it is clear the *Basella alba* is a rich source of antioxidant effect giving compounds.

Further the antioxidant capacity of all the three-plant extract was checked by DPPH method & its IC 50 value was determined. The concentration of the sample needed to scavenge 50% of the DPPH free radical using the DPPH free radical scavenging method is known as the IC50 (Half maximal Inhibitory Concentration) value. The sample's ability to scavenge free radicals or serve as an antioxidant is inversely correlated with the IC50 value. In other words, if the IC50 value is lower, the sample will need less to scavenge the free radical, and vice versa. The presence of molecules referred to as antioxidants is what causes free radicals in the sample to be scavenged.

The standard used for determining antioxidant activity was ascorbic acid. The IC 50 value for the three plants *Boerhaavia diffusa*, *Kaempferia galanga* & *Basella alba* were found to be 242.90, 249.45 & 142.55 respectively. The maximum IC 50 value of *K. galanga* indicate towards fact that it is the weakest antioxidant agent among the three plants. As the IC 50 value for *Basella alba* was lowest it can be concluded that leaves of *Basella alba* have better antioxidant potential as compared to Roots of *Boerhaavia diffusa* and Rhizome of *Kaempferia galanga*.

Table 1: Result of phytochemical screening of extract of *Boerhaavia diffusa*

| S. No. | Constituents | Chloroform extract | Ethyl acetate | Hydroalcoholic extract | Aqueous extract |
|--------|---|--------------------|---------------|------------------------|-----------------|
| 1. | Alkaloids Hager's Test: | -ve | -ve | -ve | -ve |
| 2. | Glycosides Legal's Test: | -ve | -ve | +ve | -ve |
| 3. | Flavonoids Lead acetate Test: | -ve | -ve | +ve | +ve |
| 4. | Diterpenes Copper acetate Test: | -ve | -ve | +ve | +ve |
| 5. | Phenol Ferric Chloride Test: | -ve | -ve | +ve | -ve |
| 6. | Proteins Xanthoproteic Test: | +ve | -ve | +ve | +ve |
| 7. | Carbohydrate Fehling's Test: | -ve | -ve | +ve | -ve |
| 8. | Saponins | -ve | -ve | +ve | +ve |

| | | | | | |
|----|---------------------------------|-----|-----|-----|-----|
| | Froth Test: | | | | |
| 9. | Tannins Gelatin test: | -ve | -ve | -ve | -ve |

Table 2: Result of phytochemical screening of extract of *Kaempferia galanga*

| S. No. | Constituents | Chloroform extract | Ethyl acetate | Hydroalcoholic extract | Aqueous extract |
|--------|---|--------------------|---------------|------------------------|-----------------|
| 1. | Alkaloids Hager's Test: | -ve | -ve | -ve | -ve |
| 2. | Glycosides Legal's Test: | -ve | -ve | -ve | -ve |
| 3. | Flavonoids Lead acetate Test: | -ve | -ve | +ve | +ve |
| 4. | Diterpenes Copper acetate Test: | -ve | -ve | -ve | -ve |
| 5. | Phenol Ferric Chloride Test: | -ve | -ve | +ve | +ve |
| 6. | Proteins Xanthoproteic Test: | -ve | -ve | -ve | -ve |
| 7. | Carbohydrate Fehling's Test: | -ve | -ve | +ve | -ve |
| 8. | Saponins Froth Test: | -ve | -ve | -ve | +ve |
| 9. | Tannins Gelatin test: | -ve | -ve | -ve | -ve |

+ve= present, -ve=negative

Table 3: Result of phytochemical screening of extract of *Basella alba*

| S. No. | Constituents | Chloroform extract | Ethyl acetate | Hydroalcoholic extract | Aqueous extract |
|--------|---|--------------------|---------------|------------------------|-----------------|
| 1. | Alkaloids Hager's Test: | -ve | -ve | -ve | -ve |
| 2. | Glycosides Legal's Test: | -ve | -ve | -ve | -ve |
| 3. | Flavonoids Lead acetate Test: | +ve | +ve | +ve | +ve |
| 4. | Diterpenes Copper acetate Test: | -ve | -ve | +ve | +ve |
| 5. | Phenol Ferric Chloride Test: | -ve | +ve | +ve | -ve |
| 6. | Proteins Xanthoproteic Test: | -ve | -ve | +ve | +ve |
| 7. | Carbohydrate Fehling's Test: | -ve | -ve | -ve | -ve |
| 8. | Saponins Froth Test: | -ve | -ve | +ve | +ve |
| 9. | Tannins Gelatin test: | -ve | -ve | -ve | -ve |

+ve= present, -ve=negative

Table 4: Estimation of total phenol and flavonoids content of *Basella alba*

| S. No. | Extract | Total phenol content | Total flavonoids content |
|--------|----------------|----------------------|--------------------------|
| 1. | Chloroform | - | 0.235 mg/100 mg |
| 2. | Ethyl acetate | 0.532mg/100 mg | 0.621 mg/100 mg |
| 3. | Hydroalcoholic | 0.842 mg/100 mg | 0.890 mg/100 mg |
| 4. | Aqueous | - | 0.457 mg/100 mg |

Table 5: Estimation of total phenol and flavonoids content of *Kaempferia galanga*

| S. No. | Extract | Total phenol content | Total flavonoids content |
|--------|----------------|----------------------|--------------------------|
| 1. | Chloroform | - | - |
| 2. | Ethyl acetate | - | - |
| 3. | Hydroalcoholic | 0.353 mg/100 mg | 0.756 mg/100 mg |
| 4. | Aqueous | 0.167 mg/100 mg | 0.396 mg/100 mg |

Table 6: Estimation of total phenol and flavonoids content of *Basella alba*

| S. No. | Extract | Total phenol content | Total flavonoids content |
|--------|----------------|----------------------|--------------------------|
| 1. | Chloroform | - | 0.235 mg/100 mg |
| 2. | Ethyl acetate | 0.532 mg/100 mg | 0.621 mg/100 mg |
| 3. | Hydroalcoholic | 0.842 mg/100 mg | 0.890 mg/100 mg |
| 4. | Aqueous | - | 0.457 mg/100 mg |

Table 7: % Inhibition of ascorbic acid and hydroalcoholic extracts

| S. No. | Concentration ($\mu\text{g/ml}$) | % Inhibition | | | |
|--------------|---------------------------------------|---------------|-------------------------------|-------------------------------|-------------------------|
| | | Ascorbic acid | <i>Boerhaavia diffusa</i> | <i>Kaempferia galanga</i> | <i>Basella alba</i> |
| 1 | 10 | 41.93 | 10.0 | 2.5 | 1.3 |
| 2 | 20 | 56.45 | 10.4 | 5.2 | 6.2 |
| 3 | 40 | 61.29 | 14.3 | 10.6 | 8.8 |
| 4 | 60 | 72.58 | 17.0 | 15.8 | 15.5 |
| 5 | 80 | 75.8 | 20.0 | 17.4 | 25.7 |
| 6 | 100 | 80.64 | 26.4 | 19.5 | 37.1 |
| IC 50 | | 14.23 | 242.90 | 249.45 | 142.55 |

Conclusion

In the current investigation, substantial differences in TPC, TFC, antioxidant activity, and - were found among extracts of *B. diffusa*, *K. galanga*, and *B. alba* produced using naturally occurring deep eutectic

solvents. The Hydroalcoholic extracts had the highest amount of phytochemicals present in all the three plants. The extracts made ethyl acetate had the least phytochemicals concentration. Also, TPC and TFC concentrations were found to be highest in leaves extract of *B. alba*.

Additionally, *B. alba* extracts displayed stronger antioxidant activity likely due to their higher phenolic & flavonoid content. While the antioxidant activity observed to be slightly less for *K. galangal* & *B. diffusa*. In conclusion the extracts from various parts of these three medicinal plants, can be valued highly as a source of bioactive substances and are utilized in Indian medicine to treat a wide range of diseases, would have better chances of finding use in pharmaceutical products. The study reveals that the best supplements for disorders linked to oxidative stress are medicinal plants with high antioxidant capacity. Further study at the cellular and molecular levels is required based on a thorough analysis of their chemical composition, taking into account not just polyphenols but also other phytochemicals and their in vitro toxicity potential in cell systems.

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